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Original Article

Conducting Case Study Research: A Concise Practical Guidance for Management Students

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Abstract

This article demonstrates, with real world examples, the holistic approach to case study research as a concise practical guide for management research students. It attempts to encapsulate the basic components of qualitative case study research in management studies, with special emphasis on how to set a philosophical framework, articulate research problems and theorise research results. These components of research are identified, classified, and arranged into seven pillars (7Ps) namely; Paradigm, Perspective, Purpose, Plot, Practice, Procedures, and Persuasion. Instead of following the typical sequential execution of a chain of tasks, it is continuously intermingled amongst processes by revising/redoing them to ensure more credible results and produce cogent arguments. This paper shows how to align the research context with philosophical issues (ontological and epistemological paradigm); articulate research problems and choose a methodology; theorise research findings using classical reasoning methods of abduction, deduction, and induction and; expand the theorisation beyond the original research problem. This framework would be useful to research students to place themselves in appropriate ontological and epistemological stances, eliminate doubts, enhance clarity and sharpen the focus towards plausible conclusions.

Keywords *:* Critical thinking; Methodology; Paradigm; Philosophy of research; Qualitative research.

Conducting Case Study Research: A Concise Practical Guidance for Management Students

Introduction

Publishing research results or, presenting a research project in a form of dissertation or thesis for an examination is just as important as producing research because not only for the sake of disseminating knowledge but also for the researchers' own benefits. This article demonstrates, with real world examples, the holistic approach to case study research as a concise practical guide for management research students. It attempts to encapsulate the basic components of qualitative case study research in management studies, with special emphasis on how to set a philosophical framework, articulate research problems and theorise research results.

There is little guidance on how to produce research which is consistent with ontological and epistemological assumptions throughout the research process and research students (i. e. up to PhD level) encounter many issues in terms of clarity and selection while doing research. This article attempts to demonstrate a holistic approach encapsulating the essential components of the research process, giving special emphasis on setting the philosophical framework, articulating research problems, integrating them with paradigm issues, choosing methods, and developing convincing arguments from research outcome etc. using a case-study methodology as an example. Drawing cases from a study of credit evaluation processes, it demonstrates an interactive process of articulating research problems and objectives in line with philosophical framework of critical paradigm and theorising the research findings according to the classical reasoning methods; abduction, deduction, and induction.

According to Denzin and Lincoln (2005), research methodology involves selection, justification and sequential arranging of activities, procedures, and tasks in a research project. However, instead of following the typical stages of conducting research as a sequential chain of tasks, it suggests continuous interaction between processes backward and forward by revisiting, revising and/or redoing the activities towards more credible results in order to achieve the cogent status of arguments. Yet, justification of the choice of a methodology and methods pragmatically as well as philosophically, in a research report, is important because data collection, analysis, interpretation, and conclusions drawn thereon, are considered as heavily value-laden in non-positivist paradigms. Designing and conducting a research project involve several stages from clarifying the worldviews (philosophy) of the researcher to selection of methods of gathering, analysing and interpreting data (Saunders, Lewis & Thornhill, 2007) towards conclusions.

Qualitative researchers prefer inductive, hypothesis - generating inquiry methods (over hypothesis - testing models), focus more on investigations of meaning(s) rather than behaviour and, prefer thematic analysis (Braun & Clarke, 2008). The less structured methodologies reject many of the positivists' constructions over what constitutes rigour, instead, they favour flexibility, creativity and alternative routes of inquiry that embrace storytelling, recollection, and dialogue (Parker, 2003).

Methodology

Vast literature is available for specific areas such as choosing methodologies and methods, samples and units of analysis, designing & constructing questionnaires, conducting interviews, and employing digital technology. However, not much literature is available on setting philosophical backgrounds, and how to integrate it with research problems and objectives, and also about the integrated cohesive nature of the essential components of a research project in a holistic manner. In this paper, these essential components of producing research are identified, classified, and arranged into seven pillars (7Ps) namely; Paradigm, Perspective, Purpose, Plot, Practice, Procedures, and Persuasion. This 7Ps structure shows the holistic picture of the research process in variety of contexts across several dimensions as illustrated in the Figure 1 below;

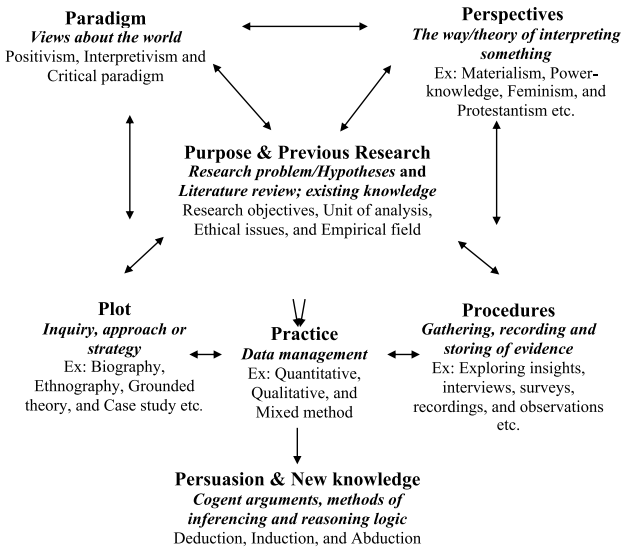

Figure 1: The seven pillars (7Ps) of case-study research structure

Source: Compiled by the author

This dynamic exercise is not a smooth process moving neatly from one stage to the other or an onion-type process which explores into the core by peeling-out layer by layer, or a tree-type process growing from ontological roots to methodological branches.

Many researchers insist that research methods and knowledge are co-evolving (Varga, 2018). In the early age of scientific research, the idealist-rationalists suggest that an absolute truth can exist uncontaminated by the experience of any observer but the more materialist-empiricists and naturalists argue that we have no ideas at all other than those which come to us via our senses (Laughlin, 1995). Later, the French philosopher Auguste Comte (1798–1857), who is generally recognised as the inventor of both 'positivism' and 'sociology' presented his thesis based on some key features such as; reality consists in what is available to senses; philosophy is parasitic on the findings of science; and there is a basic difference between fact and value, science deal with the fact and the value belongs to an entirely different order of discourse.

Anti-positivists believe that human actions are complex and have multiple meanings and argue that the concept of 'variable' used in modern quantitative analysis can only register quantifiable change, not its cause. Therefore, rather than survey-based large amount of data, anti-positivists rely on intensive studies of a small number of cases (Alvesson and Sköldberg, 2009). Critical-dialecticians such as Karl Marx (1818-1883) and non-critical interpretivists such as Max Webber (1864-1920) did not believe in any specific set of rules of governing social sciences.

Paradigms

Answers to the questions such as 'do you really know what you think you know?' and if so, 'how do you know what you know?' (Denzin & Lincoln 2005) are heavily influenced by the paradigm (set of beliefs) to which the researcher and/or the research belongs. Awareness and understanding of paradigms are vital because their underlying assumptions affect most aspects of research. Kuhn (1970) defines a paradigm as the entire set of beliefs, values, techniques that are shared by members of a community. Objectives of paradigms vary from discovering 'the Truth' to constructing/building theories in proposing solutions. Three paradigms have been widely discussed in the realm of social research namely, positivists/post-positivist, interpretivists/constructivist, and critical realism.

Paradigms compared

A positivist paradigm is associated with scientific methods where the process of discovery begins with theory, using deductive logic, and the researcher tests hypotheses from the theory (Bailey 2007). Their ontological belief is that an objective reality exists independently of any theory or human observation and can be known through research in contrast to post-positivists who concede that 'we might never know reality perfectly but ... accumulated efforts will move us toward discovering what is real' (Bailey 2007, p. 52). Contrarily, interpretivists (including constructivists) argue that the truth is constructed within the minds of individuals and between people in a culture. Similar to the interpretive paradigm, critical paradigms follow the ontological belief that there is no single reality and they stress that 'social reality is shaped by historical, social, political, cultural, and economic factors, as well as ethnic, racial, and gendered structures among others' (Bailey 2007, p. 55). The epistemological position of positivists is that knowledge which can be gained does not depend on the researcher. Positivists believe that research should be objective and value-free which means "the researcher's feelings or values should have no place in the research results"(Bailey 2007, p. 52). Objectivity, reliability, validity and generalizability are the keywords used by positivists in their vocabulary whereas anti-positivists, often guide qualitative research, may employ terms such as 'credibility', 'transferability', 'dependability' and 'conformability' (Lincoln & Guba, 2003). Further, value-neutral or value-laden anti-positivist stance is preferred in social sciences because human activity and human society are never value-free and "possibility of any unbiased objectivity no longer appeared as realistic" (Bisztray, 1987, p. 40). While positivists follow deductive methods and seek certainty, anti-positivists mainly follow inductive generalization and abductive inferencing/reasoning.

Subjectivism, objectivism and constructivism

In explaining interpretation, Umberto Eco suggests that; To interpret means to react to the text of the world or to the world of a text by producing other texts...The problem is not to challenge the old idea that the world is a text which can be interpreted, but rather to decide whether it has a fixed meaning, many possible meanings, or none at all (Eco, 1990, p. 13).

Czarniawska classifies these different schools of thought concerning the modes of explanations or interpretation into three groups: Subjectivist (voluntarist), Objectivists (determinists) and Constructivists. She claims that subjectivism is the 'most traditional way of explaining texts...by deducing the intentions of the authors...comes from reading Bible, Talmud or Koran as authored by God' (p. 63). In contrast, she suggests "the meaning of a text is neither to be 'found' nor 'created' from nothing; it is constructed anew from what already exists" (Czarniawska, 2004, p. 66). The common understanding is that researchers who subscribe to critical paradigm theory often want to document, understand and even change the way that powerful groups oppress powerless groups (Bailey 2007). The epistemological stance within the critical paradigm is that the researcher is not independent from what is researched and that the findings of research are negotiated through his or her values and desire to eradicate finance injustice (Bailey 2007). Alvesson and Skoldberg (2009) also assert that all three philosophies of science: positivism and

post-positivism, social constructionism, and critical realism cut across the quantitative/qualitative dividing line and assert that;

Although the main thrust of positivism is quantitative, there have been cases of qualitative positivism, for example historiography. Conversely, social constructionism is mainly qualitative, but quantitative social constructionist studies do exist. Finally, critical realism bridges quantitative and qualitative studies – there is no tendency for critical realists to favour either of these type of studies (p. 15).

Lincoln and Guba (2003) also stress that 'These differences in paradigm assumptions cannot be dismissed as mere philosophical differences; implicitly or explicitly, these positions have important consequences for the practical conduct of inquiry, as well as for the interpretation of findings and policy choices' (p. 112). Therefore, setting clear and strong philosophical and methodological grounds to the particular research is paramount to identify the relationship between the inquirer and the known, in selecting appropriate methods, in developing cogent arguments, and arriving at convincing conclusions. However, in the age of experimental philosophy (Anthony, 2007), it is the particular inquiry (not the inquirer) which has to be underpinned with appropriate philosophical stand-point and methodological approach in line with the context of the research. In the anti-positivist sphere, researchers choose their philosophical viewpoints and methodologies to match the nature of the research. A comparison of paradigms is presented in the following Table 1.

Conducting Case-Study Research

Table 1: A comparison of paradigms

Paradigm **Features**	***Positivist/Post-positivist***	***Interpretivist/Social constructivist***	***Critical***
Researcher	Detached observer	Attached participant	Transformative intellectual
Purpose	To discover the laws governing the universe	To understand and describe human nature	To destroy myths and change society
Objectives	Explore, explain, evaluate, predict and to develop/test theories	Understand human behaviour	Criticize social reality, liberate people, and propose solutions to social problems
Ontology	Reality or 'truth' can be known and independently exists outside of perceptions. Post-positivists concede that reality can never be known perfectly.	Reality or 'truth' is unknown and constructed within the minds of individuals. Multiple realities exist. No direct access to the real world.	Reality is created and shaped by social, political, cultural, economic forces that have been historically crystallized over time. Relativism.
Epistemology	Objective knowledge does not depend on the researcher and value-free.	Subjective perceived knowledge and value-bound/neutral; no value is wrong.	The objective-subjective label is socially contrived. Value-mediated Findings. Some value positions are wrong, and some are right.
Key concepts	Scientific, Experimental, Objectivity, Reliability, Validity and Generalizability.	Credibility, Transferability, Dependability and Conformability.	Virtual reality shaped by social, political, cultural, economic, ethnic, and gender values.
Methods	Quantitative methods are preferred. Deductive logic. Begins with theory.	Qualitative methods are dominant. Inductive generalization inferencing/reasoning. Abductive-dialectical reasoning. Hermeneutical, dialogical and dialectical.	
Theories/ Perspectives and Contributors	Rationalism: Francis Bacon (1561-1626), Rene Descartes (1596-1650) Empiricism: John Locke (1632-1704), Positivism: Auguste Comte (1798-1857) Post-positivism; Falsificationism: Karl Popper (1902-1994)	Idealism: Johann Fichte (1762-1814) Protestantism: Max Webber (1864-1920) Pragmatism: G. H. Mead (1863-1931) Symbolic interactionism: Herbert Blumer (1900-1987)	Materialism: Georg Hegel (1770-1831) Marxism: Karl Marx (1818-1883) Friedrich Nietzsche (1844-1900), Power-knowledge: Michael Foucault (1926-1984) Cultural capital: Pierre Bourdieu (1930-2002) Deconstructivism: Jacques Derrida (1930-2004)
	Idealism: Emmanuel Kant (1724-1804), Middle-range thinking: Robert Merton (1910-2003)		

Sources: Creswell, 2017; Denzin, & Lincoln, 2005; Laughlin, 1995

Perspective

Perspective is a set of rules or a theory that one applies to interpret a phenomenon. For example, in a traffic accident, the driver of one car will have one view, another driver or a passenger will have yet another view and each onlooker who witnessed the accident will have some slightly different perspectives, depending on where they were, how far they were, how good a view they had, what else was going on, how much danger they felt they were in, how the accident affected them, what the accident means to them etc. (Different perspectives, 2019). Bearing a single strong perspective might steer the researcher to snub other perspectives while holding several perspectives could make too much noise, leading to ambiguity and even some inconsistency. For example, Snyder (2015) asserts that low wage labour or sweatshops is often described as self-evidently exploitative and immoral. But for defenders of sweatshops might describe it as the first rung on a ladder toward greater economic development. On the other hand, in another perspective, it can be described as enhancing productivity or wealth maximisation which would lead to too much noise.

Purpose; research problems, objectives and literature review Often, it is the researcher's insight and experience that direct

the researcher towards a problem that needs to be researched (O'Leary, 2005). However, figuring out a problem depends on ontological and epistemological stance of the researcher therefore, chosen paradigm and theoretical perspective provide the necessary guidance to articulate a research problem. The vital-issues addressed in this process are; the curiosity, the aspirations and/or goals, appropriate research paradigms/perspectives, researchers' expertise and experience, accessibility to data, the topic, the context, the issues/questions, the motivation and potential relationship that could be explored. Articulating a reasonably acceptable research problem could stem from curiosity such as 'a burning issue', 'a mystery' or 'nice to know passion' etc., however, the lucidity of the problem and objectives largely depends on the process; an interactive exercise through initial investigations, preliminary review of literature, matching with underpinning philosophical stances, and with continuous cross-reference to the vital-issues. Application of this process is explained below using an example; a real-world experience encountered in a workplace by a researcher;

- Curiosity: Poverty, high rate of unemployment/ underemployment, income/ wealth inequality in an emerging economy.

- Aspiration: Fair and just income/wealth distribution system

This curiosity and aspiration bear an epistemological position of subjectivist value-mediated reality, stemming from an ontological position of historically crystallised situation, hence dialectical type methodology is preferred. Therefore, the suitable paradigm would be;

- Critical paradigm: Social justice and emancipation
  Capacity of the inquirer in gathering and analysing data depends largely on the expertise and exposure to the research field, for example;

- Expertise: Accounting, finance and strategic management.

- Experience: Auditing, accounting, management, and banking (treasury and credit management).

Meanwhile, review of literature reveals that;

SMEs make diverse contributions to economic and social well-being, which could be further enhanced SMEs play a key role in national economies around the world, generating employment and value added…provide the main source of employment, accounting for about 70% of jobs on average… (OECD, 2017, p.6).

- The Topic: Small and medium sized enterprises (SMEs) and employment.

According to the researchers' expertise, experience and accessibility to data;

- The context: Financial capital; loans and advances to SMEs by commercial banks in the country.

In this example, the preliminary investigations and large amount of literature revealed that there is a disadvantaged group of entrepreneurs who are deprived from credit capital; Access to the appropriate finance is one of the most crucial resources for business survival, development and growth. The literature on this is expansive and suggests that disadvantaged entrepreneurs may experience specific challenges to gaining external finance for a variety of reasons…limited know-how and network connectivity – this is compounded by relative low levels of finance capital - … disadvantaged group may be less likely to have a track record of running a business... (Blackburn and Smallbone, 2014, p. 7).

- The questions: How and why SMEs are financed. Are certain credit applicants treated favourably while some other applicants are discriminated against?

- The potential associations are identified in two aspects: (i) Social power and favourable credit decisions, and (ii) poor-powerless and denial of credit.

- The motivation: Analyse and document the factors driving discriminatory credit decisions made by credit officers in a lending institution.

Therefore, the final research problem is arrived at, as follows;

Is social power and credit approval a mutually reinforcing function while poor-powerless and denial of credit, creates a vicious cycle in the Sri Lankan society?

In other words, the purpose of this particular study is to explore the role of loan capital with regard to power relationships and access to credit for businesses in the country. In this investigation, the researcher attempts to explain how certain credit decisions are made and whether such credit decisions contribute to create a mutually reinforcing cycles and what is the impact of such credit decisions on the unemployment and poverty in the society. The following questions could also be raised as a guidance for the literature review:

(1) Are credit decisions made in favour of influential businesspeople?

(2) Are certain demographic groups at a disadvantage in obtaining credit?

(3) As a result of favourable credit decisions, could influential groups of people get richer and more influential?

(4) Are "ability to obtain credit" and "becoming more influential" mutually reinforcing?

Such multiple research questions could provide useful insights about where to look for relevant literature and evidence, especially when the researcher's approach is critical and, when structural changes are expected towards a more fair and just financial capital mobility system as the end goal (Lincoln & Guba 2003). Therefore, such research questions could provide a strong foundation to theorize the research findings more effectively and meaningfully.

This particular example research problem belongs to the critical paradigm because it focuses on critique and transformation and the issues addressed are on social power relations and inequality. Therefore, it can guide the researcher to aim at documenting, understanding and even suggesting changing the negative implications of unequal power relationships and promoting justice. The questions asked in this example research are 'why do certain bank lending processes appear discriminatory?' and 'what methods are used by the decision makers to make preferential or discriminatory credit decisions?'; therefore, the answers (and potential relationships such as 'reinforcing?' and 'co-integrated?') could be inferred from the views of research participants' experience and values.
The Figure 2 below shows a holistic picture of the research problem and potential relationships with their interconnectivity to the variables identified through the literature review.

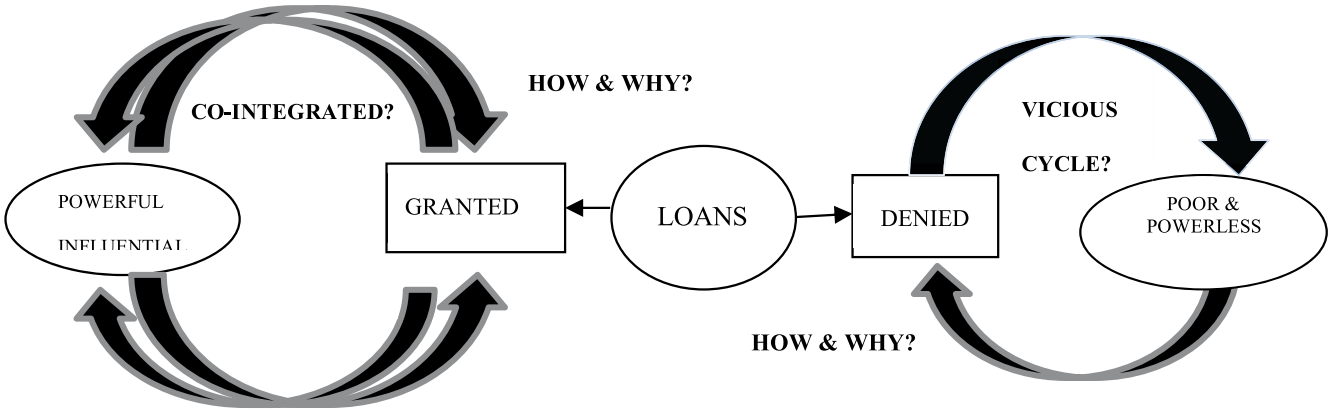

Figure 2: Illustrated Integrated Research Questions and Proposition
Source: Compiled by the author

This approach would help the researcher to review further literature, identify appropriate research fields, data gathering methods and focus on data description, analysis and interpretation (D-A-I formula of Wolcott, 1994) in developing cogent arguments and plausible conclusions.

Plot

Approaches, strategies and traditions

Creswell (2017) classifies types of inquiry into five categories namely; biography or narrative research, phenomenology, grounded theory, ethnography and case study. O'Leary (2005) suggests that 'one approach is not necessarily better than the other and methodological design is about informed decision-making that involves weighing up pros and cons and deciding what is best given your specific context' (p. 87).

Yin (1993) stresses the suitability of case study research method especially when researchers define topics broadly to cover contextual conditions (not just the phenomenon of study) and rely on multiple sources of evidence. Case study is an empirical inquiry that investigates a contemporary phenomenon within its real-life context, therefore, the case study approach is especially useful in situations where contextual conditions of the events being studied are critical and where the researcher has no control over the events as they unfold (Yin, 1993). In business and management research, case study research is considered as useful especially for practical real-world problems where experience of the actors is important and the context of the situation is critical (O'Leary, 2005). Therefore, case-study methodology seems more suitable for the research problem, objectives and questions of this particular study.

Example cases and participants

Access to data seems as an obstacle but is a critical part of conducting research (Feldman, Bell and Berger, 2003). Many scholars complain about the hardships that the researchers undergo in obtaining access to private organizations, especially banks as this could expose motives of certain powerful individuals (Lee, 2000). Therefore, obtaining access through friendships has become more common in organizational research where these barriers exist for researchers. According to O'Leary (2005), 'a prerequisite to all case selection should be access' (p. 78).

The participants of this example cases were senior bank officers having more than 20 years' banking experience and known to the researcher personally. The three cases, which have been considered for this inquiry, are typical in nature in the context of this emerging economy (why they are considered typical should also be explained in detail in the research report). Potential researchers may follow some appropriate conditional criteria, in addition to access, to select more suitable cases. For example, the Table 2 below describes conditional criteria followed to select the cases in this study. The researcher and the participants had substantial access to all the cases considered and they also complied with guidelines suggested by various scholars.

Table 2: Five conditional criteria covered by the cases selected

Table 2: Five conditional criteria covered by the cases selected

Criteria	**Cases**	**Case I**	**Case II**	**Case III**
Level of the Decision-Maker	Chairperson	√	√	
	Middle			√
Decision-making Process	Formal			√
	Informal	√	√	√
Size of the applicant	Medium	√	√	
	Small			√
Nature of the applicant	Influential	√	√	
	Not influential			√
Decisions	Credit granted	√	√	
	Credit denied			√

Source: Compiled by the author

Procedures of gathering and describing data

Procedures are the tools and techniques used for data gathering and storing. Czarniawska-Joerges (1992) uses the phrase 'insight gathering' instead of 'data collection' to encompass wider sources such as recalling memories and reconstruction of experiences. Quantitative data collection methods are much more structured than qualitative data collection methods such as interviews, open ended questionnaires, participant observations and reconstructions of experiences. Quantitative data collection methods include various forms of surveys – online surveys, paper surveys, mobile & kiosk surveys, longitudinal studies, website interceptors, online polls & forms, and systematic observations. For this example, data gathering was done through both primary and secondary sources. The primary sources are the main participants, including the researcher and secondary data consist of published documents and archival records. Data saturation is achieved by continuous gathering of data and integrating them in the analysis process in building links between events, tracing commonalities, patterns and relationships or/and posing critical questions such as 'was the decision made for personal gratification?' etc. towards interpretations and theorization. In this example, managing the three credit-client accounts for more than five years, a lot of data

was generated and the volume was adequate to achieve saturation point. Saturation of data collection can be ensured by obtaining independent feedback from peer reviewers and colleagues when there is satisfaction or deadlock of posing further why and how questions. Now it is argued that gaining traditional 'rich/thick description' alone is not enough to ensure the validity and reliability of a case study research because, it may be limited to different levels of depth and detail (Woodside and Wilson, 2003). On the other hand whether the description is 'thick' or 'thin', if it provides adequate evidence to the claim, the description is considered as dependable (Bailey, 2007).

Practices of data analysis

Practices are the choices of quantitative, qualitative or mix methods in gathering data and tools and techniques of analysis. Smith and Hodkinson (2005) point out that, 'no special epistemic privilege can be attached to any particular method or set of methods' (p. 917) but they insist that,

If the proper procedures are applied, the subjectivities (e.g. opinions, ideologies) of the knowing subject would be constrained and the knower could thereby gain an accurate and objective depiction of reality. Those researchers who adhered to method would thereby possess, in contrast to all others, what one might call the well-polished Cartesian mirror of the mind (p. 916).

Qualitative research is primarily exploratory research and used to gain an understanding of underlying reasons, opinions, and motivations. It provides insights into the problem or helps to develop ideas. Quantitative research is used to quantify the problem. It is used to quantify attitudes, opinions, behaviours, and other defined variables – and generalise results from a larger sample population. Data analysis methods for quantitative and qualitative data follow distinct strategies. They are known as statistical analysis and thematic analysis. O'Leary (2004) explains these methods as follows:

Statistical analysis – can be descriptive (to summarize the data), to inferential (to draw conclusions that extend beyond the immediate data) and,

Thematic analysis – can include analysis of words, concepts, literary devices, and/or non-verbal cues. Includes content, discourse, narrative, and conversation analysis; semiotics; hermeneutics; and grounded theory techniques (P. 11).

Thematic analysis; 'narrative analysis' (Riessman, 1993) or narrative mode of knowing also referred to as the paradigmatic mode of knowing (Bruner, 1986) seems more appropriate analytical strategy applicable to this example study because; first, as Llewellyn (1999) claims 'narrating is a mode of thinking and persuading that is as legitimate as calculating' (p. 220); second, as Czarniawska points out "the narrative mode of knowing consists in organizing experience with the help of a scheme assuming the intentionality of human action" and "'narrative' in Latin probably comes from gnarus ('knowing')" (p. 7).

Narrative approach leads to story building

The primary analysing techniques applicable within this narrative analysing strategy would be 'story building' (Riessman, 1993) and 'cross-case synthesis' (Yin, 2003) because this research is a multiple case study research. Wolcott (1994) suggests the narrative technique could be used as 'a fleshing out process of the analytical framework' (p. 21). Therefore, after data gathering (see Table 3, Description), the following questions could be raised to construct an analytical framework for those 'stories' in order to provide for more plausible and credible (Hammersley, 1992) interpretation and answering questions such as:

- Why did the borrower approach the decision-maker of the bank informally?
- Why did the top-level decision-maker accommodate the client arbitrarily?
- How and why did the middle level decision-makers approve facilities without authority?
- How and why do bankers avoid 'unimportant' credit applications?
- Why the lower-level credit officers carry out instructions coming from unauthorised sources?
- How was the transaction concluded?

Results

This analytical process supports to build the cases as stories and therefore, in this example case study research, the data description and data analysis activities are complimentarily linked together. The thick descriptions of data, detail analyses and plausible interpretations of the three case-studies mentioned above are comprised of more than 20,000 words supported with many tables as well. Therefore, it may be useful to provide a summary of the D-A-I of the cases before theorisation begins as provided in the Tables 3 below.

Table 3: Summary of the analysis of case studies data and interpretations

D-A-I		*Case Study I*	*Case Study II*	*Case Study III*
DESCRIPTION	**The Client**	A Garment manufacturing company struggling with lack of orders and liquidity problems.	An automobile assembling company, struggling with regulatory authorities and financing problems.	A janitorial-service enterprise struggling with loss of business and with financing problems.
	Credit Application	At an informal personal meeting. Both the credit decision-maker and the client are socially powerful businesspersons.	At a social meeting, outside the bank. Both the credit decision-maker and the client are socially powerful.	Formal, within the normal banking practices and credit rules. Later informal methods used by the credit decision-maker.
	Issues	Under threat of foreclosure and huge redundancy. The highest single foreign exchange earner. Affected by the recent change of regime.	Investment promotion signal. Import substitution; foreign reserves. Employment generation. Rejected registration on safety issues.	Entry of influential competitor. Soured relationship with credit officers. No access to the higher decision-making authority of the bank.
ANALYSIS	**Negotiation**	Friendly. Informal. Unofficial. Quick.		Official. Dragged.
	Decision	Without formal evaluation. Granted, over the table.		Formal granted. Formal denied. Informal granted.
INTER-PRETATIONS	**How**	'Patriotic' grounds. Enterprise promotion oriented. Without proper credit evaluation. Prevailing systems are weak. Abuse of authority. Soft application of rules.		Normal practice. Personal benefits, Error rectifying. Strict credit rules.
	Why	Known rich powerful applicants, Marxist concept of *class-consciousness.* Perceived low risk		Not influential. Powerless applicants. High risk.

Source: Saliya, 2009, 2019a, 2019b; Saliya & Hooper, 2020

Persuasion

Persuasion is the preferred method of arriving at conclusions, developing ideas and theories from research findings. Generalisation, in research, refers to extending research findings of a particular study to other settings than those directly studied. Deductive reasoning, or deduction, is used mainly in positivist-quantitative research where definite conclusions are derived. Inductive reasoning, or induction, is more uncertain and probabilistic conclusion but attempts to generalise from evidence to say something should be the case. Abductive reasoning draws inferences from observations in order to make something might be the case means conclusions may not be certain/definite. Therefore, induction and abduction belong to non-positivist (interpretivists, constructivists or critical) paradigms.

Charles Sanders Peirce (1839-1914), in his famous methodology of 'abductive inferencing' saw a way beyond inductive security of generalization and deductive certainty of derivation, as Bude (2004) describes 'deduction proves that, for logical reasons, something must be the case; induction demonstrates that there is empirical evidence that something is truly so; abduction, by contrast, merely supposes that something might be the case. It therefore abandons the solid ground of prediction and testing in order to introduce a new idea or to understand a new phenomenon' (p. 322). Bellucci and Pietarinen (2019), quoting from certain unpublished work of Peirce, reveal that Peirce (later in 1903) had argued that the three kinds of reasoning are three stages of arriving at conclusions, starting from abduction (retroduction), by which a hypothesis or conjecture is set for a deduction, which traces the outcomes of the hypothesis and finally an induction, which puts those results to test and generalizes its conclusions. Peirce took deduction to be the most secure and the least fertile, while abduction is the most fertile and the least secure (Bellucci and Pietarinen, 2019). These premises can be further elucidated with examples as shown in the Figure 3 below;

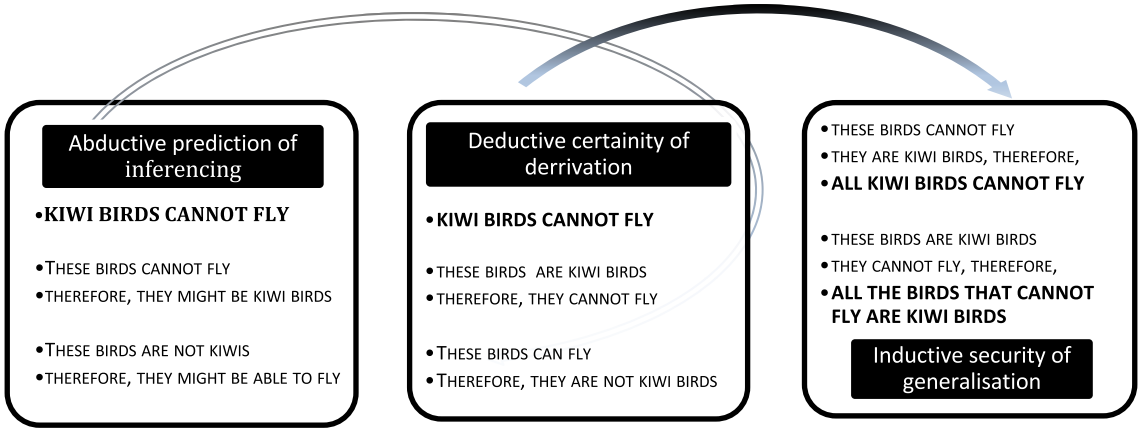

Figure 3: Abductive inferencing, deductive certainty and inductive generalisation

Source: Compiled by the author

Abductive inference suggests going beyond the data themselves and the researcher should not have to be restricted to fit themselves into existing ideas. Therefore, Abductive inference is more appropriate for qualitative inquiry, especially an open-minded intellectual approach is normally advocated. However, inductive approach is more applicable to answer 'how' questions because the conclusions are generalized from empirical evidences.
After an extensive discussion on the research results (using D-A-I formula; data description, analysis and interpretation) the following conclusions have been drawn to the example case-study research problem and potential relationships discussed above.

Theorisation

Exploring from Marxian critical theory, Lapavistas (2003) explains the motive behind such arbitrary lending decisions as follows;

Social power, privilege and inclusion in various activities are intertwined with possession of money in capitalist society. Equally, lack of money translates into powerlessness, deprivation and exclusion from several social activities for the majority of the poor in capitalism. In capitalist society, successful participation in social affairs depends less on a person's abilities and skills, and more on possession of money (p. 64).

According to Marxist theory, class-consciousness is an awareness of a social class and economic rank and their class interests, it enables members of that social class to come

together (Borland, 2008) and therefore, economically powerful social class would act together for mutual benefits. This Marxist premise 'class consciousnesses' can explain the question 'why' these powerful credit applicants were accommodated favourably (Saliya, 2019a; 2019b).

Discussion

Abductive inferences

• The economic power afforded by bank loans could eventually lead to social power and in turn, such power plays a critical role in influencing credit decision makers in the country.

• When the informal decisions involved the decision-makers at the highest level in the bank, it is more likely that the decision maker is influenced by the motive of favouritism (Marxist class-consciousness).

• Lack of money and social capital translate into powerlessness and result in deprivation of credit and could create a vicious cycle.

Advancing further in line with the researchers' curiosity and motivation, the following assertion can also be made beyond the original research problem;

• Powerful social class acquires more power through privileged credit and become more powerful. On the other hand, because the powerless entrepreneurs are neglected, opportunities could be lost to the society/country as a whole, so the poor remains poor. Therefore, socially powerful rich class get richer and richer while power-less poor class remains stagnant.

Deductive derivations

• When both credit seekers and credit decision-makers are socially powerful, credit is granted by abusing the authority overruling the normal banking practices for credit evaluation in this case.

• Powerless credit applicants are at disadvantage as they do not have access to powerful credit decision-makers in this case.

Inductive generalisations

Based on the evidences, it could be generalised that,

• Loans are approved favourably by credit officers of banks in this country when the credit applicant and/or credit decision-makers are socially powerful.

• Since the economic power afforded by money leads to social power, the socio-economic power and access to credit could form two co-integrated reinforcing functions.

• Powerless credit applicants in this country are at disadvantage as they do not have access to powerful credit decision-makers.

• Since lack of financial and social capital translate into powerlessness, credit denial and powerlessness create a vicious cycle.

Further theorisation beyond the original research problem.

In general, apart from the legitimate authority and the formal credit evaluating factors (such as risks, cash flows, feasibility and historical factors) the following factors have also been identified as critical in approving credit; the weight of socio-economic power of the parties involved, the strength of the credit policies & procedures and the vigour of the class-consciousness of the social classes. These factors are summarised and presented as a model illustrated in the Figure 4: Discriminatory credit decision-making model below.

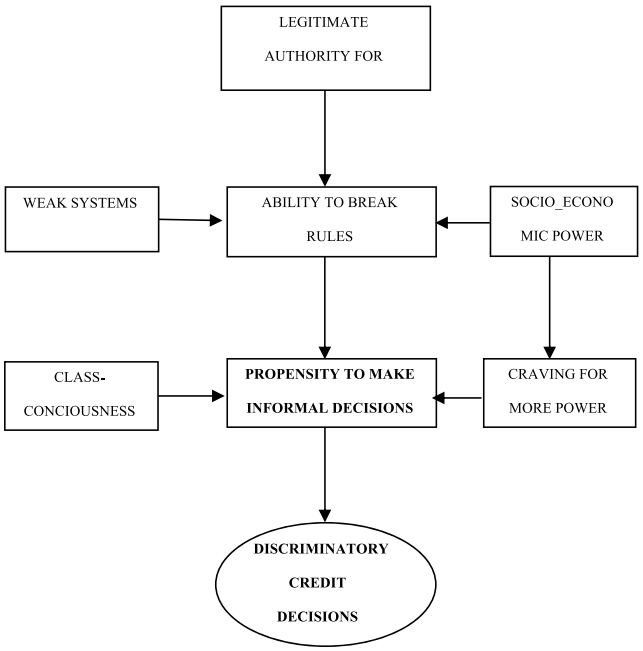

Figure 3: Discriminatory credit decision-making model
Source: Compiled by the author

Conclusions

Research methodology can no longer be confined to a set of universally applicable rules, conventions and traditions. The essential components of case-study research in management are identified, classified and, arranged into seven pillars (7Ps) namely; Paradigm, Perspective, Purpose, Plot, Practice, Procedures and, Persuasion. This 7Ps structure shows the holistic picture of research process in variety of contexts across several dimensions.

Practices and procedures are the choices of quantitative, qualitative or mix methods in gathering data but no special epistemic privilege can be attached to any particular practice or set of methods. Data analysis methods for quantitative and qualitative data are known as statistical analysis and thematic/narrative analysis.

The three kinds of reasoning are three stages of arriving at conclusions, starting from abduction (retroduction), by which a hypothesis or conjecture is set for a deduction, which traces the outcomes of the hypothesis and finally an induction, which puts those results to test and generalises its conclusions. Peirce took deduction to be the most secure and the least fertile, while abduction is the most fertile and the least secure. But abductive inference suggests going beyond the data themselves and the researcher should not have to be restricted to fit themselves into existing ideas. Therefore, Abductive inference is more appropriate, especially an open-minded intellectual approach is normally advocated. However, inductive approach is more applicable to answer 'how' questions because the conclusions are generalised from empirical evidences.

There are also gaps in guidelines provided in this paper, which should be addressed separately, on how to ensure validity and credibility in defending and generalising the research outcomes etc. Also, because many administrative protocols often seem challenging for potential case-study researchers (for example; preparing research proposals, submissions for ethical compliances and funding applications etc., if applicable) and therefore, providing further clarifications and directions in these areas would be useful to eliminate ambiguities and manage the time schedules efficiently.

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Original Article

Proportion of Three Types of Health Care Associated Infections among Hospitalized Patients in a Tertiary Care Hospital in Sri Lanka

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Abstract

Health care associated infections are considered as an indicator of quality and safety of health care institutions. The study aimed to determine the proportion of health care associated urinary tract infections, respiratory tract infections, surgical site infections, and to evaluate the association of medical devices and duration of hospitalization which predispose to health care associated infections.

Four hundred and twenty three patients who stayed more than 48 hours or readmitted to surgical, medical, pediatric and gynecology wards in a tertiary care hospital in Sri Lanka during the study period were included in the study. Data extraction sheets were used and were filled with information obtained from bed head tickets and laboratory reports.

A total of 79 (18.7%) patients had been diagnosed to have health care associated infection. The predominant type was respiratory tract infections 24 (30.4%) followed by urinary tract infections 21(26.6%), surgical site infections 17 (21.5%), and other infections 17 (21.5%). The highest proportion of urinary tract infections were seen in surgical wards (38.1%) whereas the highest proportion of respiratory tract infections were seen in medical wards (45.8%). Patients aged more than 60 were found to be affected most (27/94). Health care associated infections were significantly associated with usage of medical devices ($p<0.05$) and length of stay in hospital ($p<0.05$). The study concludes that these infections account for a noteworthy percentage in hospitalized patients and the predominant type was respiratory tract infections in this tertiary care hospital in Sri Lanka and elders were the mostly affected category compared to children and adults age groups.

Key words: HAI, TRI, UTI, SSI, Duration of hospital stay

Introduction

Surveillance of health care associated infections (HAIs) is useful in understanding the disease prevalence and to take necessary measures to minimize transmission. HAIs are considered as an indicator of quality and safety of a health care institutions1 and the Center for Disease Control and Prevention (CDC) showed that surveillance is helpful to prevent HAIs (Garner, Jarvis, Emori, Horan & Hughes, 1988).

Surgical site infections (SSI) are defined as "any purulent discharge, abscess, or spreading cellulites at the surgical site during the month after the operation. Definition for urinary tract infections (UTI) is "positive urine culture (1 or 2 species) with at least 10^5 colony forming units of bacteria / ml, with or without clinical symptoms". "Respiratory symptoms with at least two of the signs: cough, purulent sputum, new infiltrate on chest radiograph consistent with infection appearing during the hospitalization" is considered as the definition for respiratory tract infections (RTIs) (Garner et al., 1988).

In Sri Lanka few studies have been conducted to assess the HAIs in different clinical settings and shown that SSIs, UTIs and RTIs are the most common types of HAIs (Gunaratne, Vidanagama, Wijayaratne, & Palanasinghe, 2011; Gunaratne, & Vidanagama, 2011; International Committee of the Fourth International 2002). A prospective study done in the intensive care unit (ICU) at Teaching Hospital Karapitiya has reported that the main types of HAIs were ventilator associated pneumonia (VAP) (26.4%) and UTIs (10.9%) (Gunaratne et al., 2011). In 1998 Athukorala SD recorded a gradual decrease in HAI from 1994-1997 (13.5% to 8.7%) after approving and monitoring infection control measurements in Sri Lanka.

It is found that in England and Wales the proportion of MRSA isolates increased from <2% in 1990 to 42% in 2002 (Reacher et al., 2000). Prevalence of HAIs in Uganda was also found to be high (28%). Most of the HAIs surveillances were done in Western countries and a very few local studies are found (Klevens et al., 2007; Allegranzi et al., 2011; Raymond & Aujard, 2000; Cunha, 2020; Custodio, 2020).

As the data related to surveillance of HAIs in Sri Lanka is sparse, there is a greater need to investigate the health care associated infections in different clinical settings in the country. The study aimed to determine the proportion of health care associated UTIs, RTIs, SSIs, and to evaluate the association of the use of medical devices and the duration of hospitalization which predispose to HAIs in Sri Lanka. We consider that this data will help to discuss the challenges associated and opportunities to improve HAIs in the country.

Methodology

The study is a descriptive cross sectional study which was conducted in a tertiary care hospital (Colombo South Teaching Hospital) in Sri Lanka which has a bed capacity around 1000. Four hundred and twenty three patients who stayed more than 48 hours or readmitted to surgical, medical, pediatric and gynecology wards during the study period from July to August 2012 were taken as the study population".

A validated data extraction sheet was used as the study instrument. The data extraction sheets contained patients' demographic, sociologic factors, length of stay in the hospital and a check list for details of medical devices used and to diagnose three types of infections. All the data were collected according to WHO definitions of HAIs.

The proportion of health care associated UTIs, RTIs (including those recovering from VAP) and SSIs were calculated as a percentage. SPSS 16.0 software was used for data analysis. Chi square test and t- test were employed with a chosen significance level of 0.05 to describe the association of HCAIs with the usage of medical devices and the length of stay.

Results

Out of 423 patients, 203 (48%) were males while 150 (35.4%) were in surgical wards, 142 (33.6%) were in medical wards, 54 (12.8%) were in pediatric wards and 77 (18.2)% were in gynecology wards. Patients were categorized in to three age groups where 61 (14.4%) were children (age below 15 years), 268 (63.4%) were adults (16 – 60 years) and 94 (22.2%) were elders (age more than 60 years).

Seventy-nine patients (18.7 %) were identified as having HAIs. Among the patients who were diagnosed having HAIs, 24 (30.4%) had respiratory tract infections, 21 (26.6%) had urinary tract infections, 4%17 (21.5%) had surgical site infections and 17

(21.5%) other infections. Diarrhoea, sepsis and devise related infections were noted as other common health care associated infections in this surveillance.

All the patients with SSIs were from the surgical wards.

UTIs were reported from all the wards and it was highest reported in surgical wards with 8 (38.1%) whereas 7 (33.3%) was reported in medical wards, 5 (23.8%) in pediatric wards and 1 (4.8%) in gynecology wards.

RTIs were reported from all the wards, with 5 (20.8%) from surgical wards, 11 (45.8%) from medical wards, 6 (25.0%) from pediatric wards and 2 (8.3%) from gynecology wards. The highest proportion of RTIs was recorded in medical wards (Table 1).

Ward type	SSIs		UTIs		RTIs		OIs	
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Surgical	17 (100.0)	133 (32.8)	8 (38.1)	142 (35.3)	5 (20.8)	145 (36.3)	3 (17.6)	147 (36.2)
Medical	0 (0.0)	142 (35.0)	7 (33.3)	135 (33.6)	11 (45.8)	131 (32.8)	12 (70.6)	130 (32.0)
Pediatric	0 (0.0)	54 (13.3)	5 (23.8)	49 (12.2)	6 (25.0)	48 (12.0)	2 (11.8)	52 (12.8)
Gynecology	0 (0.0)	77 (19.0)	1 (4.8)	76 (18.9)	2 (8.3)	75 (18.8)	0 (0.0)	77 (19.0)
Total	17 (100.0) 21.5%	406 (100.0)	21 (100.0) 26.6%	402 (100.0)	24 (100.0) 30.4%	399 (100.0)	17 (100.0) 21.5%	406 (100.0)

Table 1: Precentage of HAIs according to types of wards

Of 79 HAI, 13 (21.3%), 39 (14.6%) and 27 (28.7%) were children, adults and elderly patients respectively according to the proportion of sample. However elderly were reported to be the most affected age group to acquire HAIs in this population. (Table-2).

Age groups	HAIs		Total	
	Yes (%)	No (%)	(%)	Infected/ Total
<15 years	13 (16.5)	48 (14)	61 (14.4)	13/61 (21.3)
16 – 60 years	39 (49.4)	229 (66.6)	268 (63.4)	39/268 (14.6)
>60 years	27 (34.2)	67 (19.5)	94 (22.2)	27/94 (28.7)
Total	79 (100.0)	344 (100.0)	423 (100.0)	(18.7)

Table 2: Proportion of HAIs according to the age groups.

According to the above findings children were more susceptible to have health care associated RTIs. SSIs, UTIs and other infections (OIs) were detected mostly in elderly patients (Table 3).

Age group	SSIs (%)	UTIs (%)	RTIs (%)	OIs (%)
<15 Years	0/61 (0.0)	5/61 (8.2)	6/61 (10.0)	2/61 (3.3)
16-60 Years	9/268 (3.4)	8/268 (3.0)	11/268 (4.1)	11/268 (4.1)
>60 Years	8/94 (8.5)	8/94 (8.6)	7/94 (7.4)	4/94 (4.3)
Total	17/79 (21.5%)	21/79 (5.0)	24/423 (5.7)	17/423 (4.0)

Table 3: Proportion of type of infection with the groups of ages.

Ninety two (21.7%) patients of the study group were connected to medical devices (Table 4) and 34 (37.0%) of them developed HAIs. Out of the patients who were not connected to any medical device 11.2% acquired HAIs. There was a statistically significant association between the prevalence of HAIs and the usage of medical devices as the chi square test was highly significant (p= <0.001).

Device usage	HAIs		Total
	Yes (%)	No (%)	(%)
Yes	42 (45.7)	50 (54.3)	92 (21.7)
No	37 (11.2)	294 (88.8)	331 (78.3)
Total	79 (18.7)	344 (81.3)	423 (100.0)

$\chi^2 = 34.3$, df = 1, p = .000

Table 4 : Association of HAIs with the usage of medical devices.

Patients who developed HAIs in this health care institution had a mean length of stay of more than 12 days (12.52), whereas those who did not acquire HAIs had a mean length of stay of less than 5 days (4.88) (Table 5). It proves that there is a statistically significant association between the length of stay in the health care setting and acquiring HAIs.

HAIs		Group statistics
Length of stay	Yes	79
	No	344

t = 5.758, df = 421, p = .000

Table 5 : Association of length of stay with the prevalence of HAIs

Discussion

Estimating the prevalence of HAIs is useful to identify the magnitude of the problem in terms of risk factors, death rates and to develop measures to mitigate the infections including outbreaks. Further, it measures the effectiveness of the infection control program employed in individual health care institutions. Several studies have been conducted in Sri Lanka related to HCAIs, and the prevalence had varied from hospital to hospital and from time to time.

Local data regarding HAI is scarce and it is proven by an article reporting that surveillance of HAI and AMR is done only in few hospitals in Sri Lanka (Jayatilleke, 2017). According to our results, the total prevalence of HCAIs was 16.8 %. A study done in intensive care presented a prevalence of HAI of 41.58% (n=42) (Pemasinghe, et al., 2012). whereas in another study, childhood healthcare associated respiratory syncytial viral RTI were detected in 86 out of total of 818 (Jayaweera & Reyes, 2019). A study conducted in Uganda showed the total prevalence of HAIs as 28% suggesting better health care in local settings . The highest UTIs percentage was recorded in surgical wards and medical wards had the highest percentage of RTI in the study conducted in Uganda (Rosenthal, et al., 2006).

A recent research conducted in Nepal reports 22.66% SSI out of 300 patients who underwent lower segment caesarean section which is significantly higher than seen in this study (Shrestha & Pradhan, 2019). Further, a meta-analysis performed covering 22 countries including Sri Lanka published a pooled period prevalence of VAP as 12.7% (Bonell, et al., 2019). The authors consider that the lower rates of their study may be due to the time it was carried out.

When comparing the prevalence of infections according to gender, females preponderance was noted in SSIs, UTIs and RTIs. However a study conducted in 55 ICUs of eight developing countries did not report any sex difference for HAI (Custodio, 2020).

When comparing the prevalence of infections with age groups, elders were the most vulnerable for HAIs. Elders were more susceptible for SSIs and UTIs and adult patients in the age group of 16-60 years were less vulnerable to UTIs when compared to the other two age groups. Further, adults and children had the highest percentage of RTIs. However, a study conducted in United States reported that RTI was most common in elderly patients and RTI and UTIs were the predominant type in children (Custodio, 2020).

In the current survey all HAIs studied (UTIs, RTIs and SSIs) were present in all the wards except in the gynecology ward. High prevalence of HAIs reported in surgical, medical and pediatric wards than in gynecology wards can be attributed to the highly crowded situation in former mentioned wards in this health care setting. Devise related infections were predominant among the category of other HAIs.

In the current study a strong statistical relationship between the usage of medical devices and the prevalence of HCAIs was seen. A previous study done at intensive care settings at Teaching Hospital Karapitiya, Sri Lanka indicated that the percentage of VAP and UTIs as 26.4% and 10.9% respectively (Gunaratne, et al., 2011) . In the current study the percentages of RTIs and UTIs were below than the above mentioned study and it may be due to less usage of devices in selected wards than in intensive care units. Another study done in developing countries during the period of 2002-2005 states that the percentage of VAP was 14.7, central venous line related bloodstream infections were 30% and catheter associated UTIs were 29% (Rosenthal, et al., 2006).

The hypothesis of association between long length of stay in the hospital and the prevalence of HAIs among hospitalized patients was significantly verified in this study. A retrospective cohort study done in China also showed that HAIs is seen with increased length of stay and thereby increasing the total cost per patient (Chen, Chou, & Chou, 2005).

Limitations

Only the patients who fulfil the WHO criteria corresponding with HAI were selected, as only symptomatic patients were valid for the study. All the patients who have readmitted due to an infection acquired at home within one week after previous discharge were considered as hospital acquired infected patients and it cannot be ascertained whether it is hospital or community acquired.

Conclusion

Based on the results of the study, respiratory tract infections were the commonest type of HAI and elderly population was affected most. HCAIs were significantly associated with usage of medical devices and with the length of the hospital stay.

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Conflict Of Interest

There are no conflicts of interests.

Ethical Statement

Ethical approval was granted from the Ethics Review Committees of Faculty of Medical Sciences, University of Sri Jayewardenepura, in 2012 and Colombo South Teaching Hospital.

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Original Article

Front Line Nurse Managers' Attitudes And Perceived Barriers Towards Continuous Education In Nursing; Sri Lanka

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Abstract

Continuous education is a vital factor which contributes towards the professional development in nursing and it is directly associated with the health of a country. Continuous education is essential to maintain and improve the knowledge, skills, and attitudes of nurses and provide better health care. The objective of this study was to assess the attitudes and perceived barriers towards continuous education in nursing among the front-line nurse managers in Sri Lanka. A descriptive cross-sectional study was conducted among 555 front line nurse managers in Sri Lanka using a self-administered questionnaire. Data analysis was done using descriptive statistics. SPSS 25 version was used as the data analysis tool. Thirty-one special grade nursing officers and 524 grade 1 nursing officers participated in this study. The majority (73%) had more than 20 years of working experience as a nursing professional. Most of the nurse managers were diploma holders (83%), 3% undergraduates, 14% graduates, and 1% were reading for Masters. Attitudes towards continuous education were good among all the nurse managers, while 92% were willing to continue their education. The majority (96%) has perceived that continuous education is vital for professional development in nursing, and 91% has stated that the opportunities and facilities for continuous education and nursing research should be improved. The attitudes borne by the front line nurse managers towards the continuous education, were considered to have been very positive. Further, continuous education was perceived by them as a vital part of professional development. Therefore, it could be surmised that the opportunities and facilities for continuous education and research on continuous nursing education should be improved in order to facilitate the provision of better health care.

Keywords: Continuous Education, Nurse Managers, Attitudes

Introduction

Education is an interactive process that increases productivity and creativity by enabling individuals to adopt new behavior throughout stages of life. The process of education empowers individuals to achieve their life goals in their discipline (Agyepong, 2018). Nursing is a scientifically rigorous discipline that regularly requires updated information to ensure that the best care is provided to patients (Agyepong, 2018) (Ekici, 2003). The continuous education of the nursing profession, with psychomotor and critical thinking skills, together has proven to be cooperative in people and society in accomplishing the health-needs. Nursing educational materials such as "nursing texts" show that continuing education is a fundamental component of nurses' professionalism. Further, continuing education can act as an organising component in the nursing function (Demiray, 2008). The nurses need to continue education as it contributes to development in the health care sector.

Nursing education has a significant impact on the knowledge and competencies of nurses. Continuous education enables nurses to cater various patients' needs, function as leaders, and contribute to advances in nursing science to deliver safe, quality patient care (Institute of Medicine, 2011). However, as adult learners, nurses ought to proceed with their learning depending on their interests and address their educational needs.

Even though nursing has been established as a profession in most of the developed nations, in Sri Lanka, it is still an emerging profession in the health service, and the range of diversity in nursing in Sri Lanka is less compared to the other developed countries. The main reason for this slow growth of the nursing profession could be due to their attitudes and perceived barriers in continuing the education process. Therefore, it is imperative to investigate the attitudes and perceived barriers for continuing education among Sri Lankan nurses in order to improve the quality of care as well as nursing as a profession.

Although the bachelor's degree in nursing commenced in the country 26 years back, the majority of frontline nurse managers hold a diploma in nursing, which specifies the need to identify the barriers in continuing education in order to address the issues immediately. Hence, this study aimed to assess attitudes and perceived barriers towards continuing education among the front-line nurse managers in Sri Lanka.

Methodology

A descriptive cross-sectional study was conducted among 555 front line nurse managers working at government hospitals in Sri Lanka. The front-line nurse managers who participated in the Annual Sessions conducted by Directorate Nursing (Medical Services) were invited to take part in the study by the investigators after providing a detailed explanation regarding the study. Front line nurse managers who attended the Annual Sessions and consented to participate were enrolled to the study. Data was collected using a self-administered questionnaire developed by the researchers using scientific literature. The questionnaire consisted of three parts; Part A: Demographic data, Part B: Attitudes related to continuing education, and Part C: Perceived barriers for continuing education. The questionnaire was pretested with the 10 front line nurse managers, and they were excluded from the main sample. The pretested questionnaire was modified based on the feedback of the participants.

Data was analysed using descriptive statistics such as frequencies, percentages, means, and standard deviations to describe the interested variables. No inferential statistics were used due to the descriptive nature of the study. Interesting findings were depicted using appropriate tables and graphs. IBM SPSS version 25 was used as the data analysis tool. Ethical approval was obtained from the Ethics Review Committee of KIU (KIU/ERC/18/019).

Results

A total of 725 front line nurse managers, participated in the Annual Sessions, and 555 front line nurse managers consented to participate in the study. The respondent rate of the questionnaire was 77% (n=555). Among them 87% were female frontline nurse managers with a mean age of 51 ± 10 years. Most of the (n=73%) nurse managers had more than 20 years of work experience, and the majority (n= 94%) were Grade I nursing officers (Table 01). The participants' educational qualifications ranged from Diploma in Nursing to reading for Master of Nursing. Among the participants 85% were Diploma holders, 15% were Bachelor of Science in Nursing graduates, and only 1% were engaged in postgraduate studies.

Table 01: Demographic Data of the study participants

Characteristics	Frequency	Percentage
Gender		
Male	71	12.8%
Female	484	87.2%
Age		
Young adult (15 – 30)	0	0%
Middle age adults (31 – 50)	246	44.3%
Senior adults (>50)	309	55.7%
Ethnicity		
Sinhala	515	92.8%
Tamil	37	6.7%
Muslim	2	0.4%
Other	1	0.2%
Designation		
Grade 1	524	94.4%
Supra grade	31	5.6%
Working Experience		
Less than 10 Years	10	1.8%
10 – 20 Years	139	25%
More than 20 Years	406	73.2%

Among the front-line nurse managers 95% of the participants, agreed that the continuous education is a vital component in nursing towards their professional development and, 93% of the participants were willing to continue their higher education. Ninety three percent agreed that research in nursing was also important for professional development, and 91% of the participants stated that the opportunities and facilities should be developed to improve continuing nursing education and research in nursing. Majority (n= 98%) of the front-line nurse managers had a good attitude towards continuous education in nursing (Table 02).

Table 02: Perception towards the continuous education (CE)

Statement	Agree %	Neutral %	Disagree %
CE is vital to professional development in nursing	96.4%	3.6%	0 %
I like to continue my education	92.4%	6.8%	0.7%
Nursing research is vital to professional development in nursing	93.2%	6.1%	0.7%
Opportunities and facilities for CE and nursing research should improve	90.5%	8.3%	1.3%
Professional Development in nursing is an important aspect of Health development	94.6%	4.7%	0.7%

Mainly three types of barriers for continuous education were identified through this study including, personal barriers, interpersonal barriers, and structural barriers. Among the personal barriers, most of the participants (n= 64%) perceived age and physical constraint as a barrier to higher education, followed by financial constraints (n=50%), domestic responsibilities (n= 44%) and time constraints (n=41%). When considering the interpersonal barriers majority perceived a lack of family support (n= 70%) as a barrier to continuous education. Among the structural barriers majority has perceived a lack of stability of the workplace (n= 62%) followed by the lack of organizational support (n= 61%) (Table 3).

Table 03: Barriers for continuous education

Type of barrier	Percentage %	
	YES	NO
Personal Barriers		
Time Constraints	41.3%	58.7%
Domestic Responsibilities	44.5%	55.5%
Financial constraints	50.3%	49.7%
Age and physical constraints	63.4%	36.6%
Interpersonal Barriers		
Lack of co-worker's support	62.2 %	37.8%
Lack of family support	70.3%	29.7%
Structural Barriers		
Work commitment	59.3%	40.4%
Lack of stability	62.5%	37.5%
Lack of organizational support of the hospital	61.4%	38.6%

Discussion

Among the front-line nurse managers, the majority were Grade I nursing officers having more than 20 years of experience in nursing practice. Despite the experience, the highest educational qualification of the majority of the front-line nurse managers was a Diploma in Nursing. However, studies conducted in neighboring countries such as India (BSc Nursing Degree - 21.2%) China (BSc Nursing Degree - 40.4%) and Australia-Sydney (BSc Nursing Degree -35.7% and Masters and PhD -2.4%) have reported greater proportions of nurses, having a bachelor's degree as their current qualification level (Naicker, 2006).

Globally, technological advancements are increasing the responsibilities in different aspects of nursing in a complex manner (Mizuno-Lewis et al., 2014). Continuing Professional Development (CPD) and Continuing Education (CE) is vital with the continuous changes which occur within the healthcare field and outside. Ninety eight percent of the frontline nurse managers who participated in this study had positive attitudes towards CE. Most of the study participants perceived that CE is a vital component in the Nursing profession. In Naicker, (2006), the work supervisors and the peers of the participants identified CE as an important aspect of nursing professional development. Nursing education is an important aspect of career development, and it has been regarded as a supplement in growth and attractiveness of the nursing profession

(Price & Reichert, 2017). The participants in the current study also claimed that nursing research is an important aspect of professional development while addressing the necessity of improving and increasing the opportunities for CE and nursing research.

Barriers to CE as perceived by the frontline nurses were categorized as personal, interpersonal, and structural. Interestingly in this study, more than half of the participants did not perceive time (n=327, 59%) and domestic responsibilities (n=311, 56%) as personal constraints of CE. However, the 'time constraint' was marked as the highest perceived personal barrier in Iranian nurses (Shahhosseini and Hamzehgardeshi, 2015). The mean age of the participants of the current study was 51+ 10 years. The results observed with regard to time and personal constraints can be expected from this population as these nurse managers have already covered most of their family commitments by this age, unlike the other nurses. Concerns and dilemmas about nursing as a profession, the lack of ability as a nursing professional, and concerns regarding developing research and further CE activities were also found as personal barriers. (Mizuno-Lewis et al., 2014). As expected, financial constraints were perceived as a confirmative drawback on CE (Fawaz, Hamdan-Mansour and Tassi, 2018; Shahhosseini and Hamzehgardeshi, 2015; Naicker, 2006). Age and physical status were reported as a perceived personal barrier in the current study (63%). Poor health or physical status was also perceived as a personal barrier but considered lesser compared to the other constraints (Naicker, 2006). When considering the frontline nurse managers with long years of experience and age, the physical status can be accepted as the most common type of personal barrier.

Lack of support from the co-workers was perceived as a constraint among more than half of the participants (62%) in the current study. Lack of family support towards CE was also higher among participants (70.3%). According to Brown (2009), lack of support from the co-workers was perceived as (58.0%), which was less than the current finding. Cooperation within the organization and outside the organization, lack of knowledge and understanding related to the organizational hierarchy and structure were also reported as the work related barriers by other reports confirming what was seen by this study (Mizuno-Lewis et al., 2014).

Overall, half of the participant's perceived structural barriers affecting the CE. Work commitment, lack of stability and the lack of organizational support were perceived by half the study participants as structural constraints for CE. Mizuno et al. in his study determined that lack of staffing, budget limitations and the relationship with the management also affect CE (Mizuno-Lewis et al., 2014). According to the literature, the structural constraints (time constraints, lack of co-workers' support and work commitments) were reported to have the highest impact based on nurses' point of view (Shahhosseini and Hamzehgardeshi, 2015; Naicker, 2006). Mid-to late-career nurses in a qualitative study claimed that CE and training were not identified as a priority by the management, and little support was provided (Price and Reichert, 2017). Organizational impact on CE and the consequences affect in terms of patient care, job satisfaction, recruitment and retention, and upgrading nursing skills (Coventry, Maslin-Prothero and Smith, 2015). Remedies to mediate the drawback in CE were discussed throughout the literature.

Continuous education for nurses needs modifications according to the necessity of the nursing learner (Eslamian, Moeini and Soleimani, 2020). Reevaluation of the current practices in CE and professional development is a must and should be encouraged (Webster-Wright, 2014). In different aspects, CE is affected, and these barriers and constraints should be addressed to maximize the retention and participation for CE. Financial support or reducing the financial burden and providing study time for CE would support the nurses to continue their studies (Lamadah and Sayed, 2014; Naicker, 2006). Offering opportunities for nurses that are reachable, convenient, and relevant to the needs of the learner would produce maximum retention and motivation among the nursing professionals (Birnie et al., 2003). These CE programs should be evaluated necessarily, to determine that the CE goals are achieved and upheld or to shift the teaching learning method of CE through evidence-based practice for better outcomes (Taylor and Hall, 2017). Governments can interfere through policy and law to uphold the CE among nursing professionals to ensure that they are recognized and considered in professional advancement (Mizuno-Lewis et al., 2014). Within the organization, there must be ways to implement financial support for CE, provide flexible times as well as to recognize the nurses who complete their advanced studies with proper remunerations (Altmann, 2012). Collaborative partnerships between educational institutions and healthcare organizations, timely and radical transformations in the curricula, increased use of simulation, inter-professional education, distance education, internships, innovative programs and many

more solutions and strategies were proposed in the literature to address the perceived barriers and negative attitudes concerning CE (Fawaz, Hamdan-Mansour and Tassi, 2018; Fitzgerald et al., 2012).

Currently, CE has become a vital requirement for meeting the international requirements to become a skillful nurse (Altmann, 2012). Therefore, the opportunities and facilities for continuous education and research in nursing should improve to provide better health care as well as uplift the professional development among nurses in Sri Lanka. While this population perceived it as a vital component, other nurses will also get the benefit of it as these frontline nurse managers would encourage their staff members to involve in the continuing education. According to the identified barriers among front line nurse managers towards continuing education can be overcome by, arranging flexible study/training programs that will match with their age, physical status and by improving the organizational support towards the nursing professionals.

Conclusion

Therefore, it can be surmised that attitudes towards continuous education among front line nurse managers were positive, and with relation to the barriers, age, physical constraints, and lack of family support were prominent among personal, interpersonal, and structural barriers. They perceived 'continuous education' as a vital part of professional development, which will have a positive impact on the nursing profession in Sri Lanka.

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Original Article

Association between urinary crystals and bacterial flora in urinary tract infection suspected patients

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Abstract

Urinary stones are an increasing clinical problem in Sri Lanka. It has been reported that 1/10 individuals experience urinary stones, yet the mechanisms responsible remain unknown. Bacteria have long been recognized to contribute to urinary crystals. However, the role of bacteria in development of urinary crystals has not been extensively investigated. This study was carried out to investigate the association between urinary bacteria flora and urinary crystals in patients with symptoms of urinary tract infections (UTI). Forty five UTI suspected patients were enrolled in the study. Mid-stream urine specimens were collected and cultured on Hi-Chrome UTI culture media. Culture plates were incubated at 37 ºC for 18-24 hours. Colony appearance and colony count were recorded and this was followed by urinary crystal count by crystal concentration technique. The Fisher's exact test was carried out to determine the association between urine crystals and presence of urinary bacterial flora. Out of 45 subjects, 29 were positive for urinary crystals, where 14 were females and 15 were males. Nineteen patients were culture positive while 18 were positive for both crystals and culture. The patients between 30-40 years showed a higher possibility to have urinary crystals and organisms. Predominant crystal type and organism detected were calcium oxalate and coliforms, respectively. Male patients in 30-40 years presented with a higher crystal concentration compared to other males. All female patients, between 30-40 years, who were positive for coliforms and presented with higher crystal concentration compared to other females in the study population. A significant association ($p < 0.001$) was observed between presence of urinary crystals and bacterial flora in UTI suspected patients.

Key words: Urinary crystals, Calcium oxalate, Coliforms, Urinary tract infections, Urine culture

Introduction

Presence of crystals in urine is known as "Crystalluria". Analysis of crystals is carried out by microscopic examination of urine. The stone formation is considered to be a medical challenge due to its multi-factorial etiology (Schwaderer and Wolfe, 2017). The etiology of urinary crystal formation is included chronic dehydration, urinary tract malformations, obstructed uropathy, metabolic diseases such as hyperparathyroidism, gout, and obesity, foreign bodies found in urinary tract and infections (Straub and Hautmann, 2005). Bacteria have been recognized as contributors in urinary crystal formation. However, the exact role of bacteria in crystal formation has not been extensively investigated though there are several findings to indicate the association between urinary crystals and bacteria (Schwaderer and Wolfe, 2017; Rahman et al., 2003; Torzewska et al., 2014). It has been noted that an elevated urinary crystal concentration, especially calcium oxalate had been recorded while performing urine full report (UFR) in routine urinalysis in Sri Lanka (Chandrajith et al., 2006; Chandrajith et al., 2019). The objective of the present study was to investigate the association between urinary crystals and bacterial flora in the urinary tract infection suspected patients.

Methods

This study is a descriptive cross-sectional study carried out among 45 patients aged 18-50 years. Patients who were enrolled at 3 private clinics with symptoms of urinary tract infections such as backache, dysuria and burning sensation while passing urine. Known UTI patients, patients with chronic diseases, antenatal mothers and urine specimens that were not delivered to the laboratory within 30 minutes of specimen collection was excluded from the study.

The study was conducted after obtaining ethical approval from the Ethical Review Committee of KIU, Battaramulla, Sri Lanka (KIU/ERC/18/049).

Data collection method

The demographic and other related data were recorded using a self-administered questionnaire.

Urine specimen collection

Urine specimens were collected from each patients who enrolled to private clinics according to the guidelines given in Laboratory Manual in Microbiology (Laboratory Manual in Microbiology Sri Lanka, 2011). Prior to collection of urine specimens, volunteers were provided with guideline which included all the necessary instructions regarding collection of clean-catch urine/mid-stream urine specimen. Volunteers were then provided with disposable, sterile, screw capped urine containers to collect urine specimens.

Urine specimen processing

The urine analysis was carried out within 30 minutes of specimen collection. Urine specimens were well mixed and inoculated on HiChrome UTI culture medium by using calibrated 0.001mL (1µL) wire loop. Inoculation was carried out according to inverted cone shaped manner and incubated at 37 °C for 24 hours. All the laboratory techniques were carried out under sterile conditions.

Reading and recording of results from culture plates growth of the colonies was observed and number of colonies were counted. Interpretation was done according to the Table 1 (Pezzlo, 1998).

Table 1: Interpretation of colony count in HiChrome UTI culture medium

Colony count	Interpretations
<10	Insignificant bacterial growth
10 – 99	Pure growth of 10^4 - 10^5 CFU/mL
More than 100	Pure growth of $\geq 10^5$ CFU/mL

Colony colour and appearance were compared with the instruction chart provided with culture medium as given in Table 2.

Table 2: Colony colour and appearance in Hichrome UTI culture medium

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
Enterococcus faecalis ATCC 29212	50-100	Luxuriant	>=70%	Blue, small
Escherichia coli ATCC 25922	50-100	Luxuriant	>=70%	Pink-purple
Klebsiella pneumoniae ATCC 13883	50-100	Luxuriant	>=70%	Blue to purple, mucoid
Pseudomonas aeruginosa ATCC 27853	50-100	Luxuriant	>=70%	Colourless (greenish) Pigment may be observed)
Proteus mirabilis ATCC 12453	50-100	Luxuriant	>=70%	Light brown
Staphylococcus aureus ATCC 25923	50-100	Luxuriant	>=70%	Golden yellow

When there was a significant growth, it was recorded with the type of organisms present and colony count.

Gram stain was then performed in order to identify the gram characteristics of the organisms.

Microscopic examination of urine sediment

Each urine specimen was poured into labeled, clean 12 mL centrifuged tubes and centrifuged at 2000 rpm for 5 minutes. The supernatant was decanted and the remaining sediment was re-suspended by adding 5 drops (0.5 mL) of supernatant onto the sediment. Type of crystals were counted in 10 high power fields and calculated in to crystal count per microliter and per milliliter of urine according to the European Urinalysis Guidelines (European Urinalysis Guidelines, 2000).

RESULTS

Demographic characteristics of study population

Gender and age distribution within the study population is given in Table 3. Majority (62.2%) of the enrolled patient population was females. Most (60.0%) of the patients were 30-39 years of age.

Table 3: Demographic chracteristics of the study population

Demographic chracteristics		Number of patients	Percentage (%)
Gender	Male	17	37.8
	Female	28	62.2
Age (in years)	20 - 29	8	17.8
	30 - 39	27	60.0
	40 - 49	10	22.2

Distribution of urinary crystals in the study population

Out of 45 subjects, 29 individuals were positive for urinary crystals whereas 16 were negative for urinary crystals. Among crystal positive patients, 15 were males and 14 were females. The types of urinary crystals present in the specimens were calcium oxalate, triple phosphate and uric acid. The number of patients with each crystal type is given in Table 4. There was no significant association ($p = 0.519$) between the age of the patients and urinary crystals (Table 5).

Table 4: Number of patients with each crystal type

Crystal type	Number of patients	Percentage (%)
Calcium oxalate	26	89.7
Triple phosphate	2	6.9
Uric acid	1	3.5

Table 5: Association between age and urinary crystals

Age groups	Patients with crystals	Patients without crystals	X^2	p value
20 - 29	4	4		
30 - 39	19	8	1.23	0.519
40 - 49	6	4		

Analysis of Hichrome UTI culture medium results

In the study population, only 19 patients had given positive results for culture; among them, 10 were

females and 9 were males. Microorganisms present were coliforms, Pseudomanas spp Staphylococcus spp. Distribution of microorganisms in culture positive cases are given in Table 6.

Table 6: Distribution of microorganisms in culture positive cases

Organism type	Male	Female	Total
Coliform spp	4	10	14
Pseudomanas spp	2	0	2
Staphylococcus spp	3	0	3

Association between urinary crystals and urine culture

Only 1 male patient with coagulase negative Staphylococcus spp was negative for urinary crystals. Other 18 patients were positive for both urinary crystals and organisms.

Eleven patients who were crystal positive, which included 4 females and 7 males, were negative for urine culture. In the study population, 15 patients including 14 females and 1 male were negative for both crystals and culture. These results have been summarized in Table 7.

Table 7: Association between urinary crystals and urine culture

	Crystal positive specimens			Crystal negative specimens		
	Female	Male	Total	Female	Male	Total
Positive cultures	10	8	18	0	1	01
Negative cultures	4	7	11	14	1	15

Both males and females in age 30-40 year group showed a higher possibility to have urinary crystals and organisms. Male patients in age 30-40 year category had a higher crystal concentration compared to others. Female patients between 30-40 years who were positive for coliforms presented with higher crystal concentration compared to other females.

According to the Fisher's exact test, there was a significant association ($p < 0.001$) between presence of urinary crystals and presence of urinary bacterial floras in UTI suspected patients as given in Table 8.

Table 8: Association between urinary crystals and urinary bacterial flora

	Crystal positive	Crystal negative	X^2	p value
Positive cultures	18	01	13.17	<0.001
Negative cultures	11	15		

Discussion

Urinary stone formation is a common disease with an increasing incidence and prevalence worldwide. This disease appears even more pronounced in industrialized countries (Daudon et al., 2004). Such observations seem to underscore the impact of lifestyle and dietary choices. According to a previous study calcium oxalate and uric acid stones are more common compared to other types (Grifth and Osborne, 1987).

In this study, 64.4% (29/45) of the UTI patients were positive for urinary crystals. Other studies have reported the prevalence of crystal formation to be about 1–5% in Asia, 5–9% in Europe, 13% in North America and 20% in Saudi Arabia (Amato, Lusini, and Nellie, 2004). In addition, the recurrence rate has been increased and exceeded 50% over a 5 to 10 year period (Bouatia et al., 2015). These reports indicate that there is a significant incidence of urine crystal formation in different populations and prevention of stone formation is of great importance to the health of general population.

According to Bouatia et al., urinary stone formation and frequency is higher in men than in women (Bouatia et al., 2015). However, an association between gender and urinary stone formation could not be found in this study as the number of males and females with urinary crystals were similar in the patient population.
Further, the same study has highlighted that the knowledge regarding mineralogical composition of urinary stones is important as it is needed to the

scientific community to explain the chemistry and the causative factors of the calculi in the urinary system (Bouatia et al., 2015). Majority of the urine specimens collected from the study population contained calcium oxalate crystals, which is similar to several reports published by other researchers (Chandrajith et al., 2019; Chandradith et al., 2006; Grifth and Osborne, 1987).

Other studies have also reported that renal stone formation and the predominant chemicals composition are age and gender dependent (Daudon et al., 2008). Most stones are formed in older patients. However, clinical observations have indicated not only a changing frequency and composition of urinary calculi but also a shift in gender and age-related incidences (Strope, Wolf and Hollenbeck, 2010). Studies have reported that children as well as adults, with factors implicated in the metabolic syndrome such as obesity now are at a higher risk for urinary stone formation (Sarica et al., 2009). However, the results of the current study found no significant association between the crystal formation and age, even though, majority of the patients with urinary crystals were between 30 – 39 years of age.

In this study, the prominent organism type was coliforms. This finding is similar to a report published by a previous study where UTI was identified to be mainly caused by E. coli (90 %), Proteus spp and Klebsiella spp (Bochud and Calandra, 2003).

The results of this study showed a significant association between urinary bacterial flora and urinary crystals. The association between bacteria and urinary crystals can be explained in different ways; bacteria may adhere to crystals leading to increased number of crystal aggregations or may bind to the tubular epithelium resulting in expression of stone matrix proteins (Schwaderer and Wolfe, 2017; Rahman et al., 2003; Torzewska et al., 2014).

Conclusion

In the present study, the main crystal type found was calcium oxalate and the predominant organism type was coliforms. There was a significant association between presence of urinary crystals and urinary bacterial flora in UTI suspected patients.

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Conflict of interest

There are no conflicts of interest.

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Original Article

Antimicrobial activity of *Rhipsalis baccifera* and *Drymoglossum piloselloides* against Methicillin Resistant *Staphylococcus Aureus* and Drug Resistant *Acinetobacter baumanii.*

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Abstract

Methicillin Resistant Staphylococcus aureus (MRSA) and multidrug-resistant Acinetobacter baumanii are known to cause delayed healing of infections in both acute and chronic wounds. Plants are a natural source of novel antimicrobials and many new drugs are derived from plants, as plants contain phytochemicals that have antimicrobial activity. Sri Lanka is a tropical country with a wide variety of plant species, many of which were identified as possessing medicinal qualities and have been used by traditional medicinal practitioners in the treatment of various diseases and ailments. Dressings made of Rhipsalis baccifera and Drymoglossum piloselloides have been used to treat wounds by Sri Lankan traditional medicine practitioners. This study determined the antibacterial activity of aqueous and methanol extracts of *R. baccifera* and *D. piloselloides* against MRSA and Multidrug-resistant *A. baumanii.* Aqueous and methanolic extractions of both plants were done by maceration. Their antibacterial properties were checked against MRSA and *A. baumanii* by the well diffusion method. The effectiveness of the extract was further tested against factors like temperature and storage time. *R. baccifera* (aqueous extract) exhibited antimicrobial properties against MRSA but no activity against *A. baumanii*. The antibiotic activity against MRSA was increased after two months of storage at 4°C. *D. piloselloides* exhibited no antibiotic activity against both MRSA and *A. baumanii.* The methanolic extracts did not demonstrate any antibacterial activity.

Keywords: Antibiotic activity, MRSA, *Acinetobactorbaumanii, Rhipsalisbaccifera, Drymoglossumpiloselloides.*

1. Introduction

The increase of antimicrobial resistance poses a very serious health threat worldwide, but an accurate net estimate of the global health burden due to antimicrobial resistance to antibiotics is unavailable (Laxminarayanan et al., 2016). New forms of antibiotic resistance can cross international boundaries and spread between continents, with ease and remarkable speed. Antibiotic-resistant microorganisms are described as "nightmare bacteria" that "pose a catastrophic threat" to people in every country in the world (WHO, 2013). When infections cannot be treated by first-line antibiotics, it is necessary to use more expensive medicines. An extended period of illness and treatment, often in hospitals, increases health care costs in addition to the economic burden on families and societies (Dijkshoorn, Nemec & Seifert, 2007). Antibiotic resistance is placing the achievements of modern medicine at risk. Chemotherapy, organ transplantations and surgeries have become much riskier without effective antibiotics for the prevention and treatment of infections (WHO, 2013).

Most bacteria can produce new strains against antibiotics and produce many virulence factors leading to antibiotic resistance which occurs when bacteria change in a way that reduces the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections (Kadurugamuwa & Beveridge, 1996).

Methicillin-resistant Staphylococcus aureus and Acinetobacter baumanii are resistant to most chemicals and drugs, such as methicilin and β-lactamase of Ampicillin (Bishburg & Bishburg, 2009) and they are frequently extremely resistant to antimicrobials, and treatment is complicated, especially for *A. baumanii*. Therefore, there is a great necessity to find new antibiotics against these two types of bacteria, which are common in wound infections (Gordon & Wareham, 2009).

Plants are a natural source of novel antimicrobials and according to the World Health Organization, 50% of new drugs are derived from phytochemicals. However, there are many plants that have not been yet investigated for antimicrobial activity. In traditional medicinal practices, plants are used in the treatment of wounds and infections. Sri Lanka, being a tropical country, has a rich diversity of plants and testing for the antimicrobial activity of the medicinal plants can lead to valuable discoveries.

Even though hundreds of plant species have been tested for antimicrobial properties, the vast majority have not been sufficiently assessed (Sangameswaran et al., 2012). The recognition of traditional medicine as an alternate form of health care and the development of microbial resistance to the available antibiotics have led researchers to investigate the antimicrobial activity of medicinal plants (Sanogo et al., 1996).

Furthermore, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that, in order to find active compounds, a systematic study of medicinal plants is vital. Many ayurvedic medicinal plants in Sri Lanka are used against wound infections (Biswas & Mukherjee, 2003). There are also many herbal plants endemic to Sri Lanka having antibacterial properties (Rajakaruna, Harris & Towers, 2002). The use of plant materials against wound infections is a very common practice in Sri Lankan traditional medicine. Considering the massive potentiality of plants as sources for antimicrobial drugs this research aims to identify the antimicrobial, activity of Rhipsalis baccifera (Kasipethi) and Drymoglossum piloselloides (Navahandi) extracts against Multidrug-resistant *A. baumanii* and MRSA.

2. MATERIALS AND METHODS

2.1 Study setting

Microbiology laboratory of the Department of Biomedical Sciences, Faculty of Health Sciences, KIU.

2.2 Study design

Descriptive study.

2.3 Collection of plant material

The plant materials that were used in this experiment, *D. piloselloides* (leaves) and *R. baccifera* (whole plant) were collected from the 'Wedagedara' Beruwala, Sri Lanka and using sealable bags all parts of plant materials were transferred to the Microbiology laboratory, KIU. Then all the plant materials were washed with tap water to remove dust and dried in shade at room temperature.

2.4 Microbiological identification of clinical isolates used in the study

2.4.1 Culture conditions and storage

All organisms were cultured on Muller Hinton Agar (MHA) and were incubated at 37°C for 24 hours. Clinical isolates of multidrug-resistant *A. baumanii* and MRSA were obtained from the University of Sri Jayewardenepura, Sri Lanka.

2.4.2 Plant Authentication

Both plants were authenticated by the Botany Division, Bandaranayake Memorial Ayurvedic Research Institute, Navinna, Maharagama. (Acc. No 2039, 2040).

2.4.3. Preparation of plant extracts

Collected plant materials were washed and dried under the shade to obtain constant weight.
Aqueous and Methanolic extracts were prepared using the maceration method.

2.4.4. Testing the antibacterial activity of selected medicinal plants against MRSA and multidrug-resistant *A. baumanii*

Both MRSA and *A. baumanii* were inoculated on a MHA plate and incubated at 37°C for 24 hours. Inoculums of 24 hours old culture was prepared in sterile saline. The turbidity of the suspensions was adjusted to the 0.5 McFarland turbidity standard. A 3 ml of bacterial suspension was added to the surface of the solidified MHA plate and spread by swirling the plate. The remaining bacterial suspension was pipetted out from the plate. A sterile pipette tip of 9mm diameter was used to cut wells on each MHA plate. The bottom of the wells was sealed by adding a drop of molten agar in the wells. A volume of 150 microliter of each concentrated plant extracts x1 (0.25mg/ml), x2 (0.50mg/ml), x 5 (1.25mg/ml) and x10 (2.50mg/ml), positive control (Gentamicin) and negative control (sterile distilled water) were loaded into wells using a micropipette. The culture plates were incubated at 37°C for 24 hours and any resulting inhibitory zones were recorded. The procedure was carried out after storage of the extracts for 1 day, 1 week and 2 months respectively. Further, the storage of the extract was done in both 4°C and -20°C to check the temperature stability.

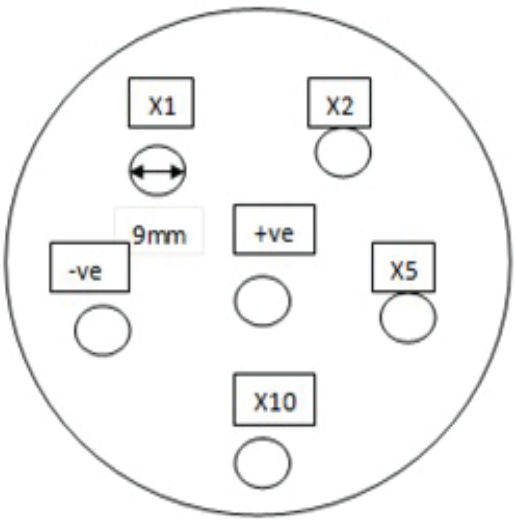


Fig 2.1; Partitioning the MHA plate for well diffusion assay.

2.5 Antibacterial sensitivity tests of bacterial strains.

In order to confirm that the collected *A. baumanii* strain is multidrug-resistant, the Antibacterial Sensitivity Test (ABST) was conducted using the disk diffusion method. *A. baumanii* was tested against cephalosporins; Ceftazidime, Cef - sulbactam - penicillin combination; ampicillin-sulbactam, Piperacillin -t azobactam -f luoroquinolones; Ciprofloxacin, Levofloxacin aminoglycosides; Gentamicin and Amikacin

2.6. Determination Minimum Inhibitory Concentration (MIC)

2.6.1 MIC by agar dilution method

For the agar dilution method, 2x concentrated aqueous extract was prepared by boiling down from 1440ml to 120ml. A concentration series of the extract was prepared by double diluting the stock solution of the aqueous extract. Molten MHA, (cooled 50°C) was added to each universal bottle to which the dilution series of the stock solution was previously added until the volume of the mixture reaches 20ml (plant extract and molten agar). Then the contents in the bottle were added to sterile Petri dishes. After the agar had solidified, the reverse sides of the plates were partitioned as shown in the figure, for the inoculation of organisms.

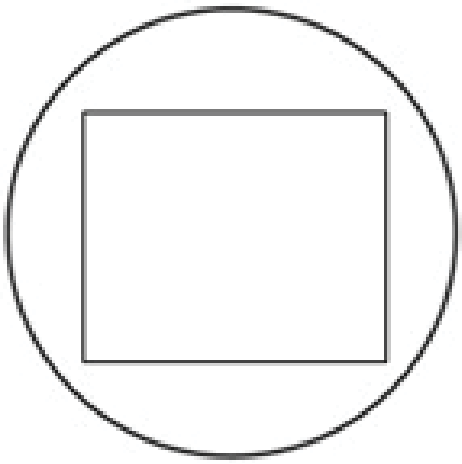


Fig 2.2: Partitioning the agar dilution plate for MIC

Next, the plates were dried at 44°C. The dried plates were inoculated with 0.5 McFarland suspension of each clinical isolate (MRSA and *A. baumanii*). The plates were allowed to stand for a few minutes to adhere to the suspension and were incubated at 37°C. The presence or absence of any growth of the organism was observed after 24 hours. The lowest concentration of extract that inhibited the visible growth of the microorganism after overnight incubation was determined as MIC. The experiment was done in triplicates and the average MIC was calculated.

RESULTS

3.1. Biochemical characterization and identification of micro-organisms.

The identity and purity of the test organisms were carried out using Gram's staining and biochemical tests. Gram's stain was carried out for both of the tested organisms at the biochemistry laboratory, KIU, Battaramulla, Sri Lanka. The test organisms considered under this research were multidrug-resistant, *A. baumanii* and MRSA (obtained from the culture collection of The Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura) which comprised of Gram-negative coccobacilli *A. baumani* and Gram-positive cocci *S. aureus*.

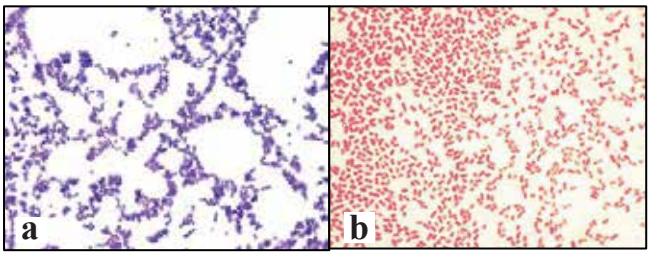


Figure 3.1: Gram characteristics of two test organisms.

(a) MRSA (b) *A. baumannii*

The biochemical characters of *A. baumanii* are shown in table 3.1. *A. baumanii* is a Gram-negative coccobacillus that is catalase positive, oxidase negative, indole, MR and VP negative and nonmotile. *A. baumanii* grew on Muller-Hinton agar, MacConkey agar, Nutrient

agar and Blood agar producing colonies of 2-3mm diameter, after 18-24 hours. The colonies produced in Muller Hinton agar were pale yellow to white greyish. Although it is known as a non-lactose fermenting organism, when grown on MacConkey agar, it fermented lactose partially with slight pink colour colonies (Figure 3.2). The colonies did not show hemolysis when grown on blood agar.

Biochemical test	Oxidase test	Catalase	Indole	Methyl Red	VP	Citrate	Urease	Haemolysis	Motility	KIA	OF
Results for *Acinetobacterbaumnnii*	-	+	-	-	-	+	-	-	Non motile	No change	Oxidative

Table 3.1; Results of the biochemical tests done for *A. baumanii*

Figure 3.2; Appearance of *A. baumanii* on MacConkey agar.

Biochemical characters of MRSA (from the culture collection of Department of Microbiology, Faculty of Medical Sciences, Sri Jayewardenepura) were tested and the results were recorded. MRSA is a Gram-positive coccus that is catalase-positive and coagulase positive.

3.2 Antibacterial sensitivity tests of bacterial strains.

In order to confirm that the collected *A. baumanii* strain is multidrug - resistant, Antibacterial Sensitivity Test (ABST) was conducted. *A. baumanii* was resistant to cephalosporins; Ceftazidime, Cef - sulbactam - penicillin combination; ampicillin-sulbactam, Piperacillin - tazobactam - fluoroquinolones; Ciprofloxacin, Levofloxacin aminoglycosides; Gentamicin and Amikacin. Figure 3.3 shows some results of ABST test done for multidrug resistant *A. baumanii.*

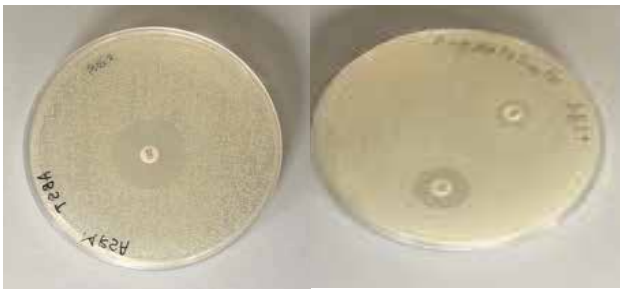


Figure 3.3; Antibiotic sensitivity tests for *A. baumanii* was done using Gentamicin, Ciprofloxacin and Ampicillin-sulbactam

3.3. Physical properties of prepared plant extracts

3.3.1 Aqueous extracts

D. piloselloides (Kasipethi) and *R. baccifera* (Navahandi) were selected to detect the antibacterial activity against *A. baumanii* and MRSA From each plant material, a concentration series was prepared as x1 (0.25mg/ml), x2 (0.50mg/ml), x5 (1.25mg/ml) and x10 (2.50mg/ml).

After preparing both plant extracts, they were filtered using a 0.22-micrometre filter. The appearance and pH of each extract were recorded (Table 3.2). The Colour of *R. baccifera* extract was yellowish-green and after boiling the clear filtered extract, a white precipitate was formed. The colour of *D. piloselloides* extract was a light green colour and no precipitate was formed upon boiling.

Concentration)	*Rhipsalis baccifera* (pH)		*Drymoglossum piloselloides*(pH)	
(mg/ml)	4^0C	-20^0C	4^0C	-20^0C
0.25 (x1)	7	7	6	7
0.50 (x2)	6	6	7	6
1.25 (x3)	7	7	6	7
2.50 (x10)	6	6	7	6

Table 3.2; pH values of the prepared concentrated extracts of each plant aqueous extracts.

3.4. Well diffusion assay

Well diffusion method was used to screen the aqueous extracts and methanolic extracts for antimicrobial activity against MRSA and *A. baumanii*. In this, any zone of inhibition observed around the well was considered as a positive response. The zones of inhibition of well diffusion assay (for both plant extracts) were obtained with respect to three variables, including concentration, storage temperature and storage time. Gentamicin was used as the positive control for both *A. baumanii* and MRSA and sterile distilled water was used as the negative control.

3.4.1. Well diffusion assay against MRSA

3.4.1.1. Rhipsalis baccifera aqueous extract against MRSA

Table 3.3 and 3.4 show the resulted mean of inhibitory zone diameter of three trial sessions conducted with respect to concentration, stored time and stored temperature.

The well diffusion against MRSA which was done one day after the preparation of the extract, (stored at 4°C) showed an average zone of inhibition (ZOI) of 13.70mm for x1 concentration, 14.70mm for x2 concentration, 15.80mm for x5 concentration and 16.80mm for x10 concentration with *R. baccifera* aqueous extract (Table 3.3). Representative plates are shown in Figure 3.4; a.
The well diffusion against MRSA which was done one week after the preparation of the extract, (stored at 4°C) showed an average zone of inhibition (ZOI) of 15.22 mm for x1 concentration, 14.44mm for x2 concentration, 15.67mm for x5 concentration and 16.67mm for x10 concentration with *R. baccifera* aqueous extract (Table 3.3). Representative plates are shown in Figure 3.3;b.

The well diffusion against MRSA which was done one week after the preparation of the extract, (stored at -20°C) showed an average zone of inhibition (ZOI) of 15.33 mm for x1(0.25mg/ml) concentration, 14.22 mm for x2(0.50mg/ml) concentration, 15.44mm for x5(1.25mg/ml) concentration and 14.33mm for x10 (2.50mg/ml) concentration with *R. baccifera* aqueous extract (Table 3.4). Representative plates are shown in Figure 3.3;c.

The well diffusion against MRSA which was carried out two months after the preparation of the extract, (stored at 4^0C) showed an average zone of inhibition (ZOI) of 13.80 mm for x1 (0.25mg/ml) concentration, 14.70 mm for x2 (0.50mg/ml) concentration, 15.80 mm for x5 (1.25mg/ml) concentration and 16.80 mm for x10 (2.50mg/ml) concentration with *R. baccifera* aqueous extract (Table 3.3). Representative plates are shown in Figure 3.3;d.

Gentamicin which was used as the positive control showed an average zone of inhibition (ZOI) of 25.00mm. (diameter of a prepared well was 9 mm.)

Stored time	Concentration	Mean diameter of the inhibition zone (mm) (extracts stored at 4^0C)
One day after the preparation of the extract	Positive control	25.00
	0.25mg/ml (x1)	13.70
	0.50mg/ml (x2)	14.70
	125mg/ml (x5).	15.80
	2.50mg/ml (x10)	16.80
One week after the preparation of the extract	Positive control	25.00
	0.25mg/ml (x1)	15.22
	0.50mg/ml (x2)	14.44
	1.25mg/ml (x5)	15.67
	2.50mg/ml (x10)	16.67
Two months after the preparation of the extract	Positive control	25.00
	0.25mg/ml (x1)	13.80
	0.50mg/ml (x2)	14.70
	125mg/ml (x5)	15.80
	2.50mg/ml (x10)	16.80

Table 3.3; Average ZOI of three trial sessions of well diffusion assay *R. baccifera* aqueous extracts (stored at 4^0C) against MRSA) that were conducted with respect to concentration and stored time.

Concentration	Mean diameter of the inhibition zones (mm) (extracts stored for one week at -20^0C)
Positive control	25.00
0.25mg/ml (x1)	13.70
0.50mg/ml (x2)	14.70
125mg/ml (x5)	15.80
2.50mg/ml (x10)	16.80

Table 3.4; Average ZOI of well diffusion assay which was done in the presence of *R. baccifera* aqueous extracts (stored at -20^0C) against MRSA with respect to concentration

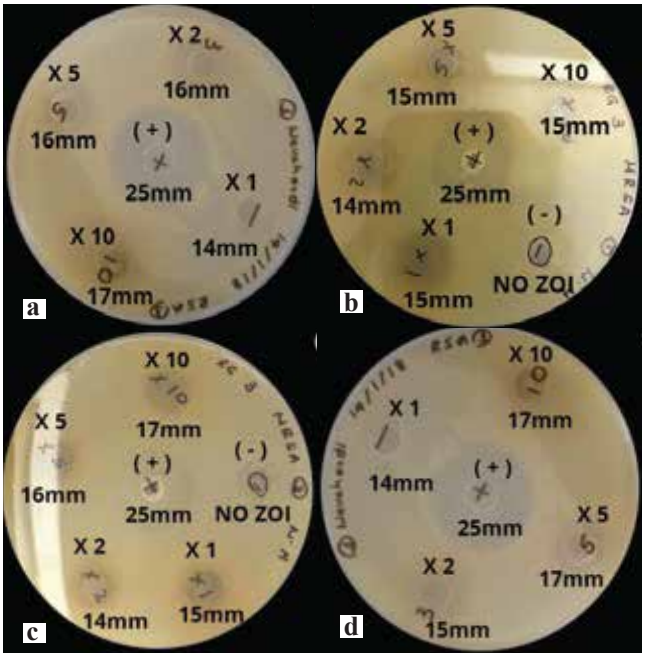
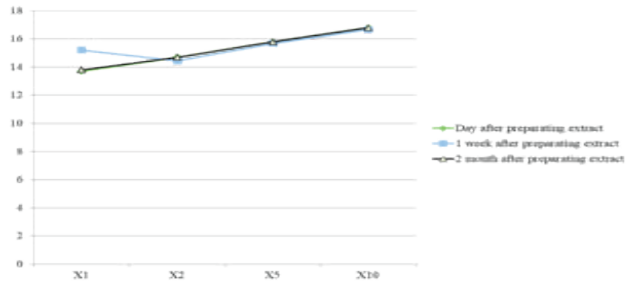


Figure 3.4; Well diffusion assay for the determination of the ZOI at different concentrations of (0.25mg/ml /x1, 0.50mg/ml /x2, 1.25mg/ml/ x5, 2.50mg/ml /x10) *R. baccifera* leaf extracts.

(a) One day after preparation (extracts stored at 4^0C) (b) one week after preparation (extracts stored at 4^0C) (c) one week after preparation (extracts stored at -20^0C) (d) Two months after preparation (extracts stored at 4^0C)



Graph 3.1 Concentration factor vs Inhibitory zones in accordance with the time stored at 4°C.

The results are shown in Figure 3.3, Table 3.3 and 3.4 depict that *R. baccifera* leaf extract (aqueous) has potential antimicrobial activity against MRSA. Furthermore, the diameter of the ZOI increases with increasing concentration. But as shown in Graph 3.1, there is no significant difference in zones of inhibition with the storage temperature and storage time in the well diffusion assay of *R. baccifera* aqueous extract against MRSA.

3.4.1.2. Drymoglossum piloselloides aqueous extract against MRSA

D. piloselloides leaf extract (aqueous) has not demonstrated any antimicrobial activity against MRSA.

3.4.1.3. Methanolic extracts of four selected medicinal plants against MRSA

Methanolic extracts of both medicinal plants have not demonstrated any antibacterial activity against MRSA.

3.4.2. Well diffusion assay against Multidrug-Resistant A. baumanii

Both aqueous and methanolic extracts of both medicinal plants *D. piloselloides* and *R. baccifera* extracts did not demonstrate any antimicrobial activity against Multidrug-Resistant *A. baumanii.*

3.5. The minimum inhibitory concentration (MIC) of tested compounds

Minimum inhibitory concentration assay was done only for *R. baccifera* against MRSA as inhibitory zones were not observed against Multidrug-Resistant *A. baumanii.* Minimum inhibitory concentration assay was not done for Drymoglossum piloselloides as no inhibitory zones were observed against both MRSA and Multidrug-Resistant *A. baumanii.*

3.5.1. MIC for Rhipsalis baccifera

The minimum inhibitory concentration of *R. baccifera* aqueous leaf extract was tested as described in the methodology and recorded as + = Growth, - = no Growth. Table 3.5 describes the MIC of *R. baccifera* aqueous leaf extract for MRSA. Representative plates are shown in Figure 4.4. The minimum inhibitory concentration of *R. baccifera* aqueous leaf extract against MRSA was x 0.5 (0.125mg/ml).

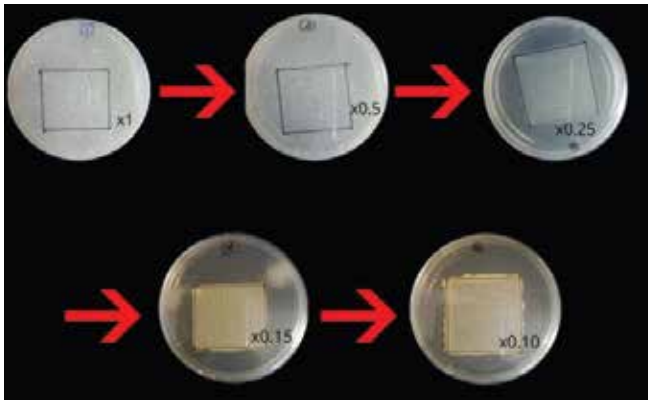


Figure 3.5; MIC assay of MRS Ain the presence of *R. baccifera* aqueous extract

Concentration	Growth of micro-organism
X1(0.25mg/ml)	-
X0.5(0.125mg/ml)	-
X0.25 (0.0625mg/ml)	+
X0.15 (0.0375mg/ml)	+
X0.10 (0.025mg/ml)	+

Table 3.5; MIC assay of MRSA in the presence of *R.baccifera* aqueous extract

4. DISCUSSION

R. baccifera is a plant widely used in traditional medicinal practices but has not been scientifically studied, so the scientific basis of its medicinal properties remain unknown.

There are many methods to prepare plant extractions to test the antimicrobial activity such as Percolation, Soxhlet extraction, Microwave - assisted extraction, Ultrasound - assisted extraction, Accelerated solvent extraction and Super rival fluid extraction. In this experiment, selected methods of extraction were aqueous and methanolic maceration extraction methods were based on the technique carried out by the traditional healer in his ayurvedic practice. These extraction methods are advantageous when considering factors like cost, and simplicity of technique.

Pre extraction techniques are performed to get a proper yield and to preserve the bio-molecules in the plants prior to extraction. The preservation methods used in the experiment were grinding and drying.

The positive control used in this experiment was Gentamicin. Even though Vancomycin is the standard antibiotic that is used against MRSA, the inability to obtain sufficient quantities of aqueous vials necessitated the selection of another option. Gentamicin was selected since it could be used against both MRSA and Acinetobactor baumanii. This plant was selected for this experiment as per the traditional healer's advice, as this is used in his practice for the healing of wounds. The results for the aqueous extract of *R. baccifera* against MRSA showed positive inhibition zones proving that it possesses antibacterial activity against MRSA. The inhibition zones increased with the increase in concentration. It is possible that this occurs due to the reason that the concentration of the active compound increases when the extract is concentrated. The initial solution produced by maceration was a clear solution, but upon boiling to prepare the concentration series, the formation of a precipitate was observed, and the amount of precipitate seen increased with the concentration. Further research is required to confirm if the formed precipitate is an active compound being crystallized. This might be advantageous for future researchers.

D. piloselloides is being used in traditional medicine for various ailments and remedies but its antimicrobial activities have also not been scientifically studied. *D. piloselloides* did not demonstrate any antimicrobial activity against both MRSA and *A. baumanii*. Further studies could be carried out testing their antimicrobial activity against other wound pathogens to determine any positive activity.

5. CONCLUSION

R. baccifera (aqueous extract) showed positive inhibition zones against MRSA which increased with the increase in concentration. It has not demonstrated any activity against Acinetobacter

baumanii.

D. piloselloides did not demonstrate any antimicrobial activity against both MRSA and *A. baumanii.*

Further studies should be carried out to identify the phytochemicals responsible for the activity of *R. baccifera* and the study could be extended to determine antimicrobial activity against other common wound pathogens. Further studies could be carried out testing the antimicrobial activity of *D. piloselloides* against other wound pathogens to determine any positive activity.

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Conflicts of Interest

None declared

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Original Article

Effectiveness of currently used urinary preservatives in preserving high demand biochemical analytes. A study in the context of Sri Lankan laboratory setting

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Abstract

Introduction

Analysis of 24-hour urine composition is widely used in diagnosis of acute and chronic kidney diseases. In Sri Lanka, no guidelines are available on preservation of urine. This study aimed to identify an effective preservation technique/s among currently practiced techniques in laboratories to maintain chemical stability of high demand bio chemical analytes in a 24-hour urine collection.

Methods

An experimental study was undertaken using urine samples from volunteers (n=42, National Hospital of Sri Lanka and University of Sri Jayawardenepura, Nugegoda, Sri Lanka). Each sample was divided in to 10 ml aliquots; Out of the lot, one was preserved without any preservative and another refrigerated at 4ºC for 24 hours. Other aliquots were preserved for 24-hours by addition of following preservatives in a 24-hour collection: boric acid g/ urine (5, 7.5, 10), sodium azide g/ urine (0.3, 0.6, 0.9), HCl ml/ urine (1N;10ml, 6N;10ml, 25ml and 30ml). The aliquots with preservatives were kept at room temperature for 24 hours. Protein, Creatinine, Ca^{2+}, Mg^{2+} and PO_4^{3-} concentrations were measured in each aliquot. Least mean squared error for each analyte in different preservatives was calculated.

Results

For protein and creatinine, least mean squared error was given by, 10g/L boric acid. That of Ca^{2+}, Mg^{2+} and PO_4^{3} were observed when the sample was refrigerated or after addition of 10g/L boric acid as the preservative.

Conclusions

Sodium azide and HCl that are utilized in current practice as preservatives for 24-hour urine collections do not show better performance in selected analytes. Boric acid (10g/L) is more effective in persevering protein and creatinine. Due to practical issues in acquiring refrigeration facilities, Ca^{2+}, Mg^{2+} and PO_4^{3-} also can be preserved effectively by using the same preservative. Consequently, Boric acid (10g/L) could be recommended as an effective preservative to preserve selected analytes in 24-hour urine collection.

Keywords: Urine preservatives, 24-hour urine collection, Boric acid, Creatinine.

Introduction

Analysis of 24-hour urine has been widely used to focus on an appropriate basis for the decisions on the diagnosis of acute and chronic kidney diseases (Kumar & Clark, 2017). This is due to the fact that urine reveals a wide spectrum of diagnostically important biochemical information about the human body (Kumar & Clark, 2017; Burits & David, 2014). Therefore, accuracy and reliability of the routine laboratory investigations based on the methodological standardization of patient preparation, sample collection and preservation of 24-hour urine samples are important components of the preanalytical stage of the laboratory testing and reporting (Feres et al., 2011). In this context, accurate collection methodologies of any biological fluid are very important to avoid pre analytical errors and recurrent sample collections. Due to the prolong sample collection time, minimization of variations in urine composition is critically important to maintain the accuracy of laboratory investigations (Burits & David, 2014).

Protein, creatinine, pH, electrolytes, inorganic ions (Na^{+}, K^{+}, Cl^{-}, Ca^{2}+, Mg^{2+},HCO^{3}-, PO_4^{3-}, heavy metals (mercury, zinc, nickel), urea, amino acid, cortisol, cysteine, oxalate and uric acid are analytes that can be tested using 24 hour urine sample (Feres et al., 2011).

Bacterial action, chemical decomposition and atmospheric oxidation of unstable compounds are some problems encountered with periodical collection. Conversion of urea into ammonia by bacterial action gives unpleasant ammonical odour and causes difficulties in determination of urea, ammonia, pH and total nitrogen (Burits & David, 2014). Further bacteria utilize creatinine for their growth and convert it into creatine which may lead to underestimation of the test results (Burits & David, 2014).

The consistency of the biological sample is a major factor for optimal laboratory results. Therefore, various preservatives and preservation methods are used based on their physiochemical roperties in determination of biochemical analytes.

Refrigeration, use of HCl, boric acid, sodium azide, formaldehyde, thymol and toluene are commonly used preservation methods to inhibit degradation and to preserve urinary analytes (Thongboonkerd & Saetun, 2007). Refrigeration minimizes the rate of bacterial action and chemical decomposition (Burits & David, 2014). Boric acid has a bactericidal action against most urinary bacteria (Kumara et al., 2015; Meers & Chow, 1990). Though sodium azide is toxic to humans, it has a bacteriostatic effect by inhibiting cytochrome oxidase in gram negative bacteria (Walsh et al., 2003). Thymol also has an antibacterial activity because of it's phenolic structure (Iqbal et al., 2015).

Selection of an appropriate preservative depends on the analyte that needs to be investigated. Although different strategies have been reported in the literature, the effectiveness in preservation of biological analytes is questionable. Feres et al., (2011) conducted a clinical trial using 24-hour urine samples of 22 volunteers, which were collected in HCl as the preservative under three conditions. According to the results, it has been concluded that protein did not show accurate values in the presence of acid preservatives and analytes which require acid preservatives (creatinine) showed acceptable values in acidification. Ferraz et al., (2006) carried out a study on 34 healthy subjects and preserved 24-hour consecutive duplicate urine samples (pre delivery acidified samples and post-delivery acidified samples) with 6 mol HCl to analyze Ca^{2+}, oxalates, uric acid and creatinine. Same procedure was carried out with spot urine samples with HCl and $NaHCO_3^{-}$. The results revealed that there was no significant difference between pre and post-delivery acidification results. This made the conclusion that acidification is not needed for the preservation of above mentioned analytes.

Another study was conducted by Sodi et al., (2009) to identify the necessity of acidification of urine before measuring Ca^{2+}. The study recruited 133 patients to collect paired 24-hour urine samples. Samples were preserved with 5 mol/L HCl and the Ca^{2+} levels obtained were compared with that of unpreserved urine in the same pair. The results suggested that acidification was not necessary at pre analytical stage to measure Ca^{2+}.

A study was carried out to evaluate the chlorhexidine/n - propyl gallate as a urine preservative using pooled urine to detect long term storage ability (Nillen & Smith, 2004). It was found that the mixture had the ability to store urine without changing several parameters including Ca^{2+} and creatinine.

Yilmaz et al., (2008) investigated the necessity of a preservative in collection of 24-hour urine samples. Twenty-four hour collections (n=50) and spot urine samples (n=20) were preserved with HCl and $NaHCO_3^-$. Results showed that addition of a preservative was not necessary at pre analytical stage to measure Ca^{2+}, Mg^{2+}, PO_4^{3-} and uric acid. A case study reported that accidental ingestion of sodium azide in 24-hour urine container resulted in cardio vascular collapse and death (Herbold et al., 1995). Therefore, it was concluded that refrigeration is more suitable to avoid accidental ingestion of harmful preservatives. Bacteriostatic effect of boric acid was evaluated in another investigation and the results showed that 10g/L boric acid is weakly bactericidal and 10 -20 g/L boric acid is more effective (Meers & Chow, 1990). In this context the studies which have been conducted until now, don't focus on providing any recommendation on superior methods to preserve urine for investigation purpose of biological analytes.

However, due to low temperatures and favorable climate in western countries, urine samples are stored at room temperature in which the analytical procedures are carried out. Even though storing urine samples at room temperature is recommended for some analytes in international guidelines, it is difficult to adopt in Sri Lanka due to temperature variations that favours the bacterial growth. When urine samples are stored at room temperature, the abnormal ammonical (pungent) odour comes out from older urine samples due to conversion of urea into ammonia by the action of bacteria (Burits & David, 2014). It may cause difficulties in carrying out analytical procedures. In turn, refrigeration could not always affordable In turn, poor communities in Sri Lanka cannot afford refrigeration despite its wider applicability in other countries.

Measurement of protein and creatinine concentrations in 24-hour urine collection is predominant in urinalysis. Nevertheless, in Sri Lankan laboratories, several protocols for urine analysis have already included vast variety of preservatives to prevent physiochemical and biological changes during urine collection (Table 1) whereas some others have not. Unavailability of a single preferred method to preserve biochemical analytes in a 24-hour urine sample is a major drawback in this line of investigations. Therefore, at present the patient is required to collect multiple urine samples for different analytes. Also, a scientific study has not been conducted in Sri Lanka to support or to make any recommendations on methods to be adopted to preserve a 24-hour urine sample. In this context, standardizations for urine collection and appropriate sample preparation methods are, therefore, crucially required.

Thus, this study aimed to identify an effective preservation technique/s among currently practiced techniques in chemical pathology laboratories to maintain chemical stability of high demand bio chemical analytes (e.g. protein, creatinine, calcium, magnesium and phosphorus) in a 24-hour urine collection. Further an attempt was taken to acclaim an appropriate method/s to minimize patient discomfort in collection of multiple samples for different analytes. Preservatives for the study were selected from the currently available list used in chemical pathology laboratories of Sri Lanka.

Table 1: In-use preservatives for 24-hour urine collection in Healthcare Institutions, Sri Lanka

Analyte to be preserved	Preservative
Protein	98% HCl 5g Boric acid 10g Boric acid 10% Thymol (2 ml) 10 g Thymol Few crystals of Thymol 10% Thymol (1 ml) 10% Thymol (10 ml) in isopropanol 0.5 g Sodium azide Toluene 30 ml
Creatinine	6 mol/HCl (10 ml) 6 mol/HCl (25 ml) 98% HCl (10 ml) Concentrated HCl (10 ml) 6 N HCl 30 ml 10% HCl 10 ml 20 % Acetic acid (20 ml) 10g Boric acid 5g Boric acid 10% Thymol (2ml) in isopropanol 10% Thymol (10ml) in isopropanol Toluene (30 ml)
Ca^{2+}, Mg^{2+}, PO_4^{3-}	6 mol/HCl 10 ml, 6 mol/HCl 25 ml 98% HCl 10 ml Concentrated HCl 10 ml 6 N HCl 30 ml 10g Boric acid 20 % Acetic acid (20 ml) Toluene (30 ml) Few crystals of sodium azide Toluene 30 ml 10% Thymol 10ml in isopropanol Few crystals of sodium azide

Materials and Methods

Subjects

The study was conducted at the chemical pathology laboratory of the National Hospital of Sri Lanka. Forty-two (42) participants were enrolled after obtaining the written consent. The study was ethically approved by the Ethics Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura (Protocol approval No. MLS 11/2015) and Ethics Review Committee, National Hospital of Sri Lanka. Patients who were already diagnosed with acute or chronic kidney diseases in renal unit and nephrology clinic of National Hospital of Sri Lanka and age-related healthy volunteers from university population were included in the study. Study population was selected to represent normal and high values of urinary analyte concentrations anticipated to measure.

Methods

Each sample was divided into 12 aliquots, each containing 10 ml urine as detailed in the Figure 1. First sample was tested immediately without preservatives for protein, Ca^{2+}, Mg^{2+} and PO_4^{3-}. Second sample without any preservative was stored at 4^0C (refrigerator) for 24 hours. The other 10 aliquots were preserved for 24 hours at room temperature after addition of preservatives. Preservatives were selected based on currently in-use preservatives of chemical pathology laboratories of Sri Lanka (Table 1). Samples were analyzed for protein, creatinine, Ca^{2+}, Mg^{2+}, PO_4^{3-} post preservation of 24 hours. A detailed schematic representation of the methodology is depicted in Figure 1.

The results were analyzed by using SPSS to derive mean squared error of each analyte in different preservatives in comparison with that of unpreserved sample (freshly voided urine sample). The preservation method which had the least mean squared error was considered as the best preservation method (among the other tested methods) for the corresponding urinary analyte.

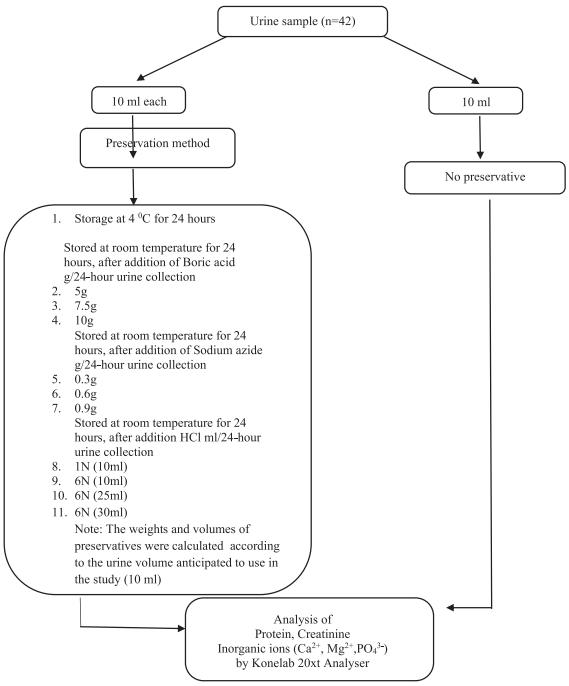

Figure 1: Schematic summary of the study design adopted in the present study

Results and Discussion

Urine may be a waste product, but it contains an enormous amount of information. Well-standardized procedures for collection, transport, sample preparation and analysis should become the basis of an effective diagnostic strategy for urinalysis. As reproducibility of urinalysis has been greatly improved due to recent technological progress, preanalytical requirements of urinalysis have gained importance and have become stricter. Since the patients themselves often sample urine specimens, urinalysis is very susceptible to preanalytical issues. Various sampling methods and inappropriate specimen transport can cause important preanalytical errors. The use of preservatives may be helpful for particular analytes. Unfortunately, a universal preservative that allows a complete urinalysis does not exist yet. In Sri Lanka different preservatives are used to preserve 24-hour urine collections as shown in table 1. According to the current practices adopted in Sri Lanka, analysis of high demand biochemical analytes requires collection of multiple samples which is inconvenient to the patients. The aim thereby was to select an ideal preservative which can be used to preserve most analytes to avoid discomfort to the patient.

According to the present study, the least mean squared error for protein and creatinine was permitted when urine was preserved with 10 g boric acid / 24-hour urine collection (Table 2). Therefore, among other methods, addition of boric acid (10 g) was moderately effective in preserving proteins and creatinine, though this method was not used in most of the Sri Lankan government institutions. Addition of HCl as the preservative for urinary protein showed the highest mean squared error. However, it was the method that is in practice at some institutions.

The findings of the present study are supported by a similar study conducted by Feres et al., (2011) concluding that analysis of microalbumin and protein in 24-hour urine samples did not show good performance in the presence of acid preservatives.

The present findings are inconsistent with the study carried out by Ferraz et al., (2006) indicating that acidification is not necessary for creatinine assay. Further, the present study revealed that the refrigeration did not show better performance for analysis of protein and creatinine in 24-hour urine samples.

The study suggests that, for the analysis of inorganic ions, the most effective preservation method was refrigeration at 4^0C due to the lowest mean squared error observed (Table 2). A similar study has been carried out by Yilmaz et al., (2008) and supports the findings of the present analysis indicating that no preservatives are needed for the assay of inorganic ions in 24-hour urine samples. The next lowest mean squared error was obtained by 10g boric acid / 24-hour collection for inorganic ions (Table 2). The same preservative showed the best preservation performance with protein and creatinine in the present study. In some communities of Sri Lanka refrigeration facilities are not available due to economic hindrances. Therefore, 10g boric acid / 24 hour collection may have the potential to preserve urinary inorganic ions also, effectively. Since sodium azide has toxic effects and also proven to have a higher mean squared error, the present study does not recommend sodium azide as a preservative in 24-hour urine collections, though it is currently used in different institutions in Sri Lanka (Herbold et al., 1995).). To the best of our knowledge, this data set is the first that provides the direct evidence of effectiveness of chemical stability on the urinary analyte profile. Our present study has addressed an important issue of “sample collection and storage” prior to the analysis of high demand biochemical analytes in urine.

Table 2: Mean squared errors of each measured analyte in urine when preserved in different preservatives (in comparison with analytes in freshly voided urine)

Preservative / Analyte	Storage at 4°C	1N HCl 10ml	6N HCl 10ml	6N HCl 25ml	6N HCl 30ml	Boric Acid 5g	Boric Acid 7.5g	Boric Acid 10g	Sodium Azide 0.3g	Sodium Azide 0.6g	Sodium Azide 0.9g
Protein	71.4888	78.3831	73.6648	58.6461	73.6499	32.4121	48.9231	31.3597*	76.2053	42.6796	71.0604
Creatinine	0.1071	0.0857	0.0760	0.0779	0.4448	0.2083	0.0955	0.0662 *	0.1679	0.0895	0.3179
Ca^{2+}	0.0045 **	0.0096	0.0060	0.0079	0.0272	0.0173	0.0056	0.0050 ***	0.0072	0.0251	0.2244
Mg^{2+}	0.0169 **	0.0279	0.0237	0.0196	0.0190	0.0218	0.0284	0.0194 ***	0.0251	0.0236	0.0274
PO_4^{3-}	0.1812 **	0.3358	1.9729	0.7964	0.2988	0.4583	0.3248	0.2481 ***	0.4490	0.7455	0.5831

*Least mean squared error for protein and creatinine; Boric acid 10g

**Least mean squared error for Ca^{2+}, Mg^{2+} and PO_4^{3-}; Refrigeration

***Least mean squared error next to refrigeration for Ca^{2+}, Mg^{2+} and PO_4^{3-} ; Boric acid 10g

Conclusion

On the basis of the data reported herein, our recommendation is to preserve protein and creatinine by addition of boric acid 10g /1L, 24 hour urine collection. Even though, other inorganic ions (Ca^{2+}, Mg^{2+} and PO_4^{3-}) are more preserved in refrigerated samples, boric acid 10 g/L in 24-hour urine collection may be used as most suitable preservative with better preservation ability of all the analytes in a urine sample. Further, this can be implemented even in a peripheral setup where there are no refrigeration facilities. When a single preservative is used to preserve multiple analytes that have been ordered by the clinician, the patient needs to collect a 24-hour urine sample only once. This will omit the necessity of collection of several 24-hour collections with different preservatives as in current practice. This will minimize the discomfort which afflicts patient and is more important in critical care patients.

Conflicts of interest

There is no conflict of interest.

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