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RESEARCH ARTICLE

IMPLEMENTATION OF PARETO PRINCIPLE IN IDENTIFICATION PROGRAM OF BACTERIAL ISOLATES IN HEALTHCARE ENVIRONMENT

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Abstract

Background and aims: Healthcare associated infections are a major concern, causing an estimated 72,000 deaths and costing the United States up to $45 billion annually. This study aimed to identify and characterize bacterial isolates in healthcare facilities to improve infection control.
Methods: Using biochemical identification and Pareto analysis, samples were examined to determine the most prevalent bacterial species. The Pareto principle helped focus efforts on the major contaminants.
Results: Results showed that *Pseudomonas* genus (34.6%) and *Micrococcus genus* (19.2%) were the most abundant, accounting for more than 50% of isolates. The presence of multiple bacterial species, including both Gram-positive and Gram-negative bacteria, suggests widespread prevalence, likely a result of inadequate cleaning and contamination of water or surfaces. Gram-positive bacilli were less common due to their lower environmental resistance.
Conclusions: The study concluded that implementing proper cleaning and disinfection protocols and regularly monitoring water quality are essential for preventing cross-infection and ensuring a safe environment. Identifying the most prevalent bacteria using the Pareto principle is a crucial step in mitigating the risk of microbial contamination.
Keywords: disinfectant validation, healthcare-associated infections, *Micrococcus* spp., Pareto principle, *Pseudomonas* spp.

INTRODUCTION

Nosocomial infections, also known as healthcare-associated infections (HAIs), can have significant costs in terms of human life, health, and finances[1,2]. The impact of HAIs includes loss of life, adverse health outcomes, and significant strain on healthcare systems and finances[1,2]. According to the Centers for Disease Control and Prevention (CDC), HAIs are responsible for an estimated 99,000 deaths per year in the United States alone[2]. While this mortality rate according to CDC's 2015 database has declined later to 72,000, HAIs lead to longer hospital stays, increased healthcare costs, and a higher risk of complications such as surgical site infections (SSIs)[1]. From an economic perspective, the total cost of HAIs in the United States ranges from $28 billion to $45 billion per year, which includes direct treatment costs and indirect costs from lost productivity and disability[3]. Furthermore, HAIs increase the demand for resources and the workload for healthcare workers, which can negatively impact the quality of care, patient outcomes, and satisfaction[1,3]. Preventing and controlling these infections is essential to improving patient outcomes, reducing healthcare costs, and maintaining community health.

The risk of bacterial infection and cross-contamination is a major concern in hospitals, leading to numerous nosocomial and surgical site infections[4]. These are caused by bacteria such as *S. aureus*, *E. coli*, and *P. aeruginosa*[5]. To minimize this risk, healthcare facilities must implement strict protocols for hand hygiene, environmental cleaning, and equipment sterilization[6]. Regular surveillance, staff training, and targeted interventions are essential components of an effective infection control program[7-10].

Biochemical identification of bacteria from environmental sources is critical for detecting potential pathogens and preventing their spread. Identifying the specific species present in sources like water, cleaning materials, and on surfaces helps healthcare workers select the most effective infection control measures[4,11]. By isolating and identifying bacteria, medical professionals can take appropriate actions, such as changing cleaning solutions or implementing specific precautions to prevent transmission between patients[4,7]. While microbial monitoring is standard practice, the application of systematic management tools like the

Pareto principle to prioritize environmental bioburden is not well-documented.

Therefore, the present work aimed to screen samples from a selected healthcare facility to evaluate microbial identification profile based on the identified bacterial populations and to take further protective measures such as applying disinfectant validation programs on the isolates of concern through future studies.

MATERIALS AND METHODS

Microbiological sample collection, transportation and analysis

Sampling microbiological specimens from healthcare facility environments in Egypt, water sources, and equipment cleaning efficiency tests was conducted to screen for bacterial isolates through isolation, microscopical examination and biochemical identification[12]. Specimen handling followed standard microbiological procedures[15]. The sampling strategy was planned to determine critical areas, the number of samples, and the methods to be used, such as swabbing for surfaces or sterile bottles for water. All sampling equipment was sterilized via autoclaving or 70% alcohol to prevent contamination. Samples were collected from high-touch surfaces, water distribution points, and other areas of interest according to the plan. Collected samples were transported to the laboratory in cool conditions as quickly as possible to prevent changes in the microbial population. Laboratory analysis was performed using appropriate methods, such as agar surface inoculation or membrane filtration, to identify and quantify bacterial species. The study implemented the Pareto concept as a supportive technique to help healthcare professionals identify major microbial contributors[13,14]. The general procedural steps are detailed below.

Bacterial isolation and gram stain

The process involved several basic steps[16-18]. Samples from sterile swabs or containers were used to inoculate prepared agar plates via a streaking technique to isolate individual bacterial colonies. Plates were incubated at appropriate temperatures and durations to allow for bacterial growth. Isolated colonies were selected for further testing. Gram staining was performed using a standard four-reagent kit (crystal violet, iodine, alcohol, safranin) to differentiate bacteria based on cell wall structure[19,20]. Stained slides were examined under a microscope at 100x and 1000x magnification to observe bacterial morphology and arrangement.

Application of biochemical identification system

A rapid, automated biochemical identification system was used to identify bacterial isolates based on biochemical tests and computerized algorithms. The general steps for using the system for Gram-positive and Gram-negative isolates were as follows[21,22]. A pure bacterial isolate was grown on an agar plate and transferred to a biochemical card, which was then loaded into the instrument. The instrument performed a series of tests for enzymatic activity or metabolic reactions to determine the isolate's identity. Results were confirmed with additional tests, such as a manual Gram stain (as mentioned previously), particularly for unexpected identifications. All results were recorded in a laboratory information management system (LIMS).

Data interpretation and Pareto analysis

Pareto Principle (80/20 rule) was applied to focus on the most abundant identified bacteria and hence the possible sources. The practical application involved identifying the “vital few” (the 20% of bacterial types causing 80% of contamination), prioritizing control measures on these key factors, and regularly evaluating the impact of these interventions[23-25]. To account for the unequal number of samples across sources (Water n=31; Environmental n=14; Cleaning Efficiency n=10), a 'Corrected Abundance' metric was calculated to account for variation due to variable sample sizes from each source.

RESULTS AND DISCUSSION

Proper microbiological sampling in hospital environments is a critical component of maintaining hygiene and preventing the spread of infectious diseases[26-28]. In this study, analysis of samples from a healthcare facility revealed a nearly equal distribution between Gram-negative rods and Gram-positive cocci (Figure 1). However, the distribution varied by sources of contamination (Figure 2). Pareto analysis of the identified isolates revealed that two genera, *Pseudomonas* and *Micrococcus*, were the most significant contributors to the facility's overall microbial bioburden showing 53.8% by genus (Figure 3, Figure 4) and Pseudomonadota phylum contributed by more than 50% in this study.

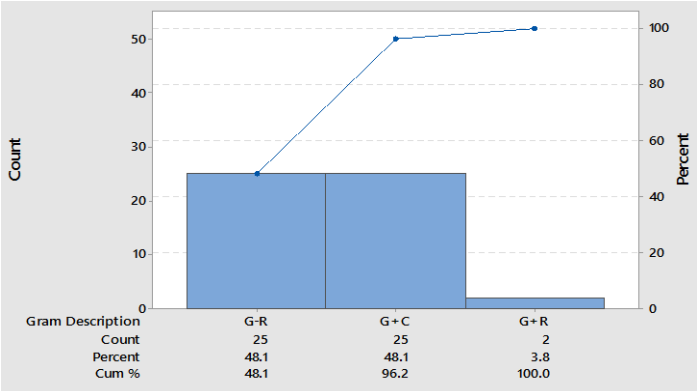

Figure 1: Pareto distribution of screened bacteria as Gram stain general morphology.

Table 1: Identification distribution profile of the isolated bacteria from healthcare facility.

Source	Sample (n)*	Identification	Frequency	Gram Description	Frequency of Gram Type	Sample Fraction Contribution	Relative Sample Abundance %	Relative M.O. Abundance %	ID/Sample Type	Corrected Abundance
Water	31	*S. anginosus*	4	G+C	25	0.596	59.62	7.69	2.00	3.35
		S. maltophilia	4	G-R	25	0.596	59.62	7.69	4.00	6.71
		Pediococcus species	2	G+C	25	0.596	59.62	3.85	2.00	3.35
		S. schleiferi	2	G+C	25	0.596	59.62	3.85	2.00	3.35
		P. fluorescens	6	G-R	25	0.596	59.62	11.54	6.00	10.06
		P. aeruginosa	10	G-R	25	0.596	59.62	19.23	8.00	13.42
		C. diphtheriae	2	G+R	2	0.596	59.62	3.85	2.00	3.35
		S. capitis	4	G+C	25	0.596	59.62	7.69	2.00	3.35
Environmental	14	*Micrococcus* species	2	G+C	25	0.269	26.92	3.85	2.00	7.43
		M. lylae	6	G+C	25	0.269	26.92	11.54	4.00	14.86
		M. luteus	2	G+C	25	0.269	26.92	3.85	2.00	7.43
		S. anginosus	4	G+C	25	0.269	26.92	7.69	2.00	7.43
		S.carnosus	2	G+C	25	0.269	26.92	3.85	2.00	7.43
		S. capitis	4	G+C	25	0.269	26.92	7.69	2.00	7.43
Cleaning Efficiency	10	*Myroidesodoratus*	2	G-R	25	0.192	19.23	3.85	2.00	10.40
		P. putida	2	G-R	25	0.192	19.23	3.85	2.00	10.40
		P. aeruginosa	10	G-R	25	0.192	19.23	19.23	2.00	10.40
		M. lylae	6	G+C	25	0.192	19.23	11.54	2.00	10.40
		S. epidirmidis	2	G+C	25	0.192	19.23	3.85	2.00	10.40

Abbreviations: M.O., Microorganism; G-R, Gram-negative rod; G+C, Gram-positive cocci; G+R, Gram-positive rod.
*Unknown or unidentified samples (n = 3) were excluded from this analysis and hence from the calculations.

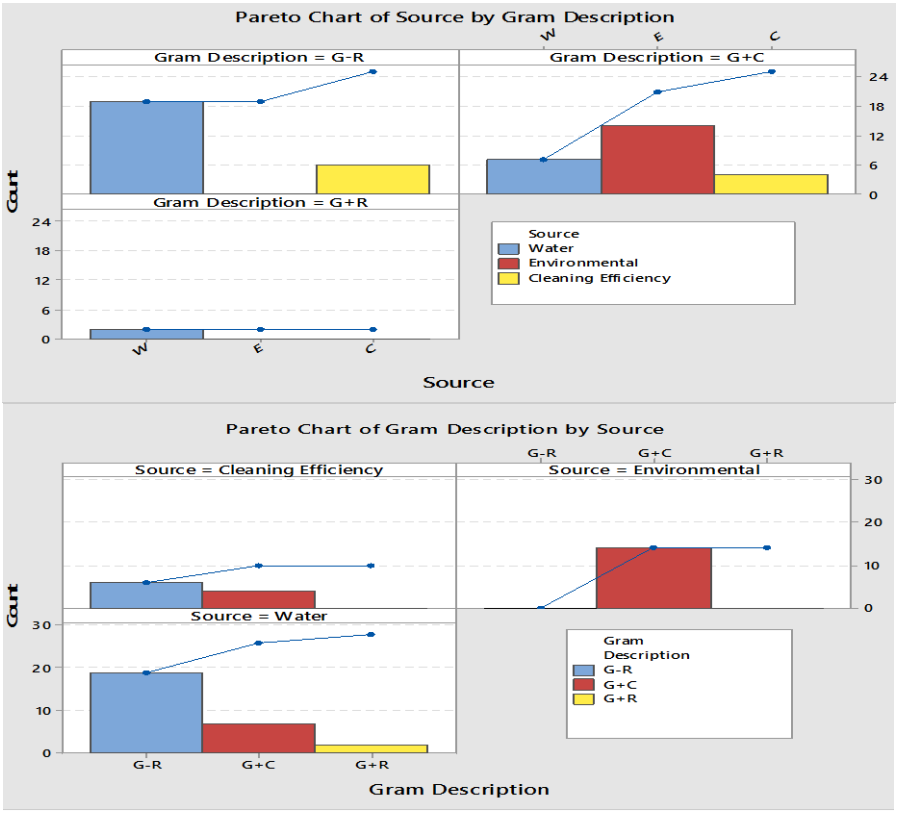

Figure 2: Pareto analysis of bacterial distribution in the sample types by Gram stain general morphology.

As shown in Table 1, *Pseudomonas spp.* (*P. aeruginosa*, *P. fluorescens*, *P. putida*) and *Micrococcus spp.* (*M. lylae*, *M. luteus*) were frequently isolated. The types of bacteria identified varied significantly by sample source (Figure 2, Figure 5). Gram-positive cocci such as *Staphylococcus* and *Streptococcus spp.* were primarily found in environmental and cleaning samples. In contrast, Gram-positive rods were less abundant overall. Isolating a toxigenic *C. diphtheriae* is a major public health event. Notably, three species *Myroides odoratus*, *P. fluorescens*, and *S. epidermidis* were isolated exclusively from cleaning efficiency

samples. The finding of a nearly equal distribution between Gram-negative rods and Gram-positive cocci suggests mixed contamination from multiple sources. Such a scenario can arise from cross-contamination, where bacteria are transferred between sources, such as from medical equipment to water systems[29]. The identification of *Pseudomonas* and *Micrococcus* as the most significant contributors is a key finding for prioritizing infection control measures. *Pseudomonas* is a ubiquitous Gram-negative bacterium found in soil, water, and vegetation, and is a known opportunistic pathogen in hospital water systems[11, 30]. *Micrococcus* is a Gram-positive bacterium common in air, soil, and water and can colonize areas with poor ventilation; while often non-pathogenic, it can cause infections like endocarditis in immunocompromised patients[31].

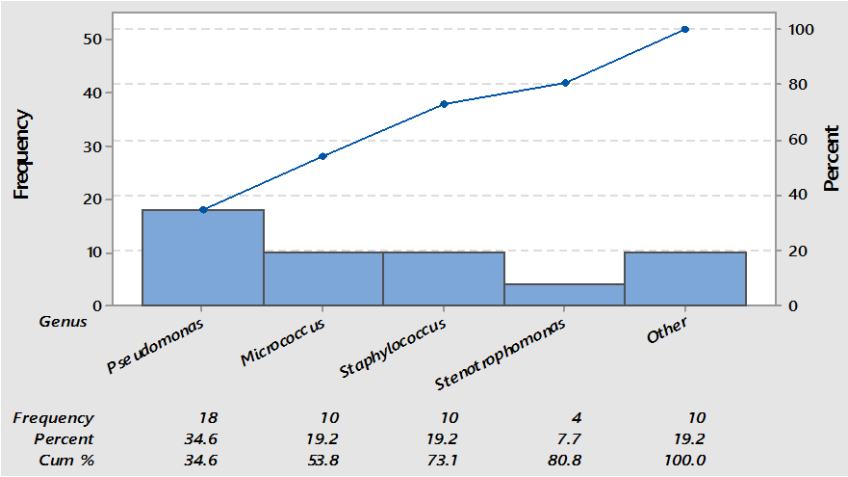

Figure 3: Pareto analysis of bacterial distribution in the sample types by genus.

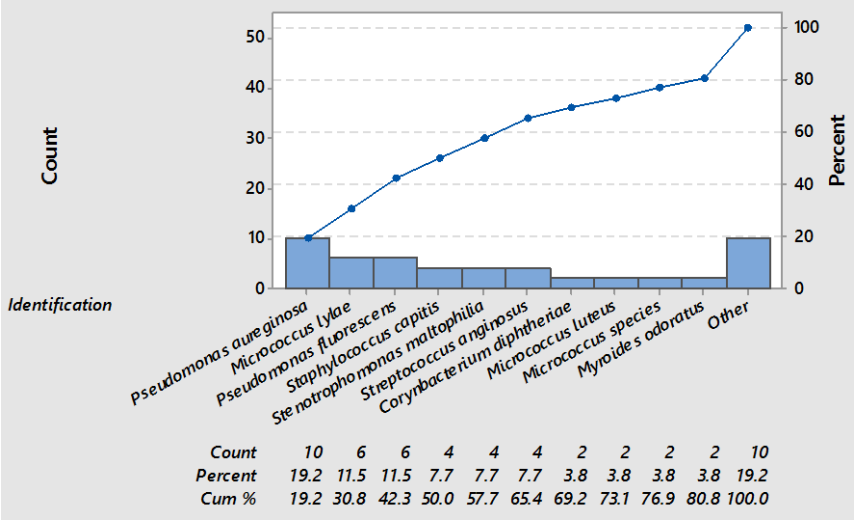

Figure 4: Pareto chart showing a descending order of the identified bacterial species by their abundance and frequency of detection.

The prevalence of these organisms highlights their environmental resilience and underscores their importance as primary targets for infection control and cleaning validation programs.

The distribution of microorganisms across different sources provides further insight. The prevalence of *Staphylococcus* and *Streptococcus* in environmental and cleaning samples is expected, as these bacteria are common colonizers of human skin and can persist on dry surfaces, spreading through contact with contaminated people or equipment[4,7,11]. The lower abundance of Gram-positive rods can be attributed to their lower resistance to environmental stresses and disinfectants compared to cocci and Gram-negative rods[32]. The presence of Gram-positive rods like *Corynebacterium diphtheriae* is often linked to contamination from soil, sediment runoff, or biofilm formation within water distribution systems[33,34].

The exclusive isolation of *Myroides odoratus*, *P. fluorescens*, and *S. epidermidis* from cleaning efficiency samples is significant, as these organisms can serve as indicators of inadequate cleaning and disinfection protocols. All three are common environmental bacteria found in soil and water that are known to survive on surfaces for extended periods, even after cleaning procedures[35-38]. Previous studies have identified the persistence of these specific bacteria on hospital surfaces post-cleaning, suggesting their utility as markers for evaluating cleaning efficacy[39-43]. Their presence does not necessarily indicate a direct health risk but rather reflects a procedural weakness that requires attention.

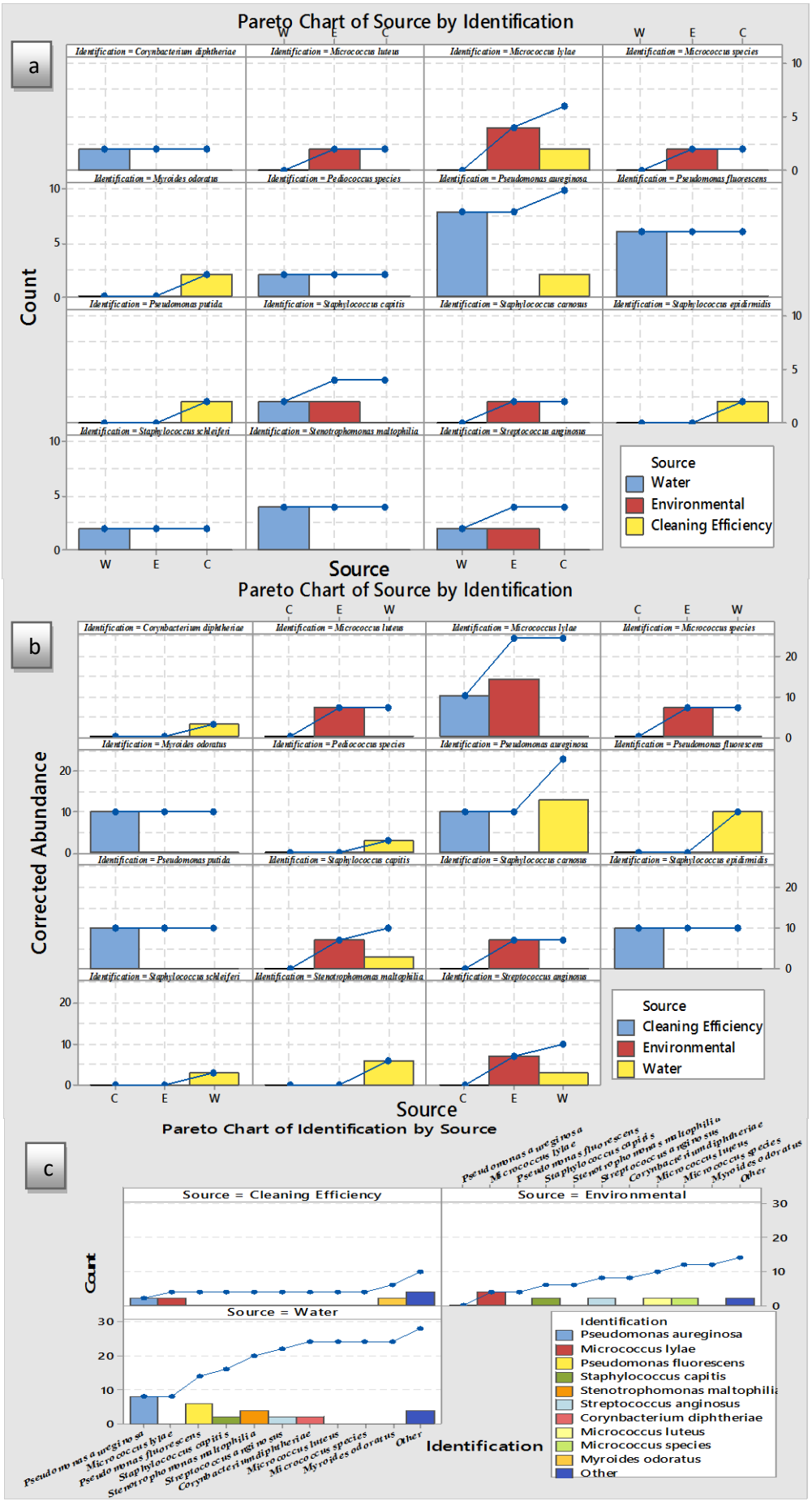

Figure 5: (a) Distribution profile of each identified bacteria in the sample types for the healthcare facility. (b) Corrected distribution profile of each identified bacteria in the sample types for the healthcare facility. (c) Collective distribution of the identified bacteria by the sample types in the healthcare facility.

Effective cleaning protocols, regularly monitored, are essential to remove these resilient bacteria and prevent their potential to cause infections in immunocompromised patients[44].

Limitations of the study

While this study provides valuable insights into the application of the Pareto principle for bacterial surveillance, several limitations should be acknowledged to contextualize the findings. The scope of the research was confined to a single healthcare facility, and the sample sizes, particularly for environmental and cleaning efficiency sources, need expansion in future screening studies; therefore, the results may require complementation from other settings and should be validated across a broader range of locations. The reliance on biochemical identification, though standard for routine diagnostics, could be complemented in future work by molecular methods to confirm species-level identification with higher resolution. Furthermore, the cross-sectional nature of the sampling offers a snapshot of microbial prevalence, and longitudinal studies would be beneficial to understand temporal fluctuations and the long-term impact of targeted interventions. Finally, while the corrected abundance metric was applied to account for uneven sample sizes, the inherent variability between source types suggests that findings related to less abundant species should be interpreted with caution.

CONCLUSIONS

The presence of an even distribution of Gram-positive cocci and Gram-negative rods indicates that a healthcare facility is experiencing microbial contamination from multiple sources, requiring a comprehensive investigation to identify specific contamination pathways. Application of the Pareto principle successfully identified *P. aeruginosa* and *S. aureus* as the most important targets for improved monitoring and control. Furthermore, the exclusive presence of organisms such as *Myroides odoratus* in cleaning efficiency samples highlights their value as practical indicators for verifying the effectiveness of cleaning and disinfection protocols. Regular monitoring of cleaning efficiency and water quality, based on these findings, is essential to prevent bacterial persistence and spread and ensure a safe healthcare environment.

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None to declare.

AUTHOR'S CONTRIBUTION

Eissa ME: designed the study, performed the statistical re-analysis, manuscript writing, microbiological interpretation, critically reviewed.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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