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Research Article

The Prevalence and Automated Identification of Microorganisms Isolated from Clinical Samples in Kalar City

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ABSTRACT

Effective isolation and accurate identification are two critical steps in diagnosing the causes of microbial infections. The ability to effectively analyze and identify isolates using both manual isolation and automated instruments is vital to the identification process. This study investigated the prevalence and distribution of bacterial and fungal isolates from various clinical samples. Laboratory experiments and surveys were conducted among patients who attended Zanko Medical Lab in Kalar City from August 2022 to March 2024. A total of 691 bacterial strains were isolated, with a high identification rate of 94.9%, using the automated VITEK 2 Compact system for microbial identification. Gram-negative bacteria constituted the majority (59.04%), followed by Gram-positive bacteria (34.15%) and yeasts (1.75%). *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were the most common Gram-negative isolates from urine, with *E. coli* and *K. pneumoniae* being more frequent in females and *P. aeruginosa* being more frequent in males, particularly those undergoing endoscopic surgery. Among Gram-positive isolates, *Staphylococcus haemolyticus*, *Enterococcus faecalis*, *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Staphylococcus saprophyticus* were commonly identified, primarily from urine specimens. Urine samples constitute (84.2%) of the isolates, high vaginal swabs (7.9%), sputum samples (2.9%), and other samples (4.9%). The VITEK 2 Compact system is crucial for accurately identifying most isolates of gram-negative and Gram-positive bacteria. Some are too difficult to identify using different methods, such as semi-automated approaches.

Keywords: Prevalence, isolation, microbial identification, VITEK 2, clinical isolates

INTRODUCTION

Isolation and identification, two critical steps in diagnosing the causes of microbial infection, are not without challenges. However, with their unparalleled expertise, professional microbiological lab technicians play an essential role in overcoming these challenges. The ability to effectively analyze and identify isolates using both manual isolation and automated instrumental approaches is outstanding and vital to the process. The challenges posed by the complexity and diversity of microbial communities are still difficult, even with the introduction of advanced technologies. This is where skilled microbiological laboratory technicians come into play, as they perform both old-fashioned and automated work equally well and ensure accurate pathogen identification.[1]

Gram-negative bacterial infections pose a common problem and concern internationally, with a wide range of these infections representing a significant majority of cases, between seven to nine cases out of every ten infected patients.[2] The pathogens are most frequently encountered in order of their occurrence, starting with *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, followed by some Gram-positive bacteria such as *Staphylococcus saprophyticus*, *Enterococcus faecalis,* and Group B *Streptococcus*, which pose threats to the vulnerable population and contribute significantly to the infections.[3]

Global healthcare-associated infections rank urinary tract infections (UTIs) on top, with 35% of all infections being UTIs. The difficulty arises in the diagnosis and management of UTIs due to the asymptomatic nature of infections and the multilayered interactions of different microbiome infections worldwide. The asymptomatic nature and complications of microbiome interaction at the points of infection make diagnosis and management of UTIs an exceptionally difficult task.[4,5]

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Microbes can now be identified accurately with improved devices like the VITEK 2 Compact system. This system has been shown to have become better in the detection of both common and rare pathogens, which in turn, improves overall infection control.[6,7] It is essential in detecting multidrug-resistant microorganisms as a predominant threat to global health.[8]

The study aimed to analyze the frequency and distribution of microbial isolates from clinical samples collected in Zanko Medical Lab in Kalar City. Advanced automated systems were used to identify the pathogens, including the most biochemical results in 24 h, assisting in spreading enhanced strategies for antimicrobial therapy aimed at combating antibiotic resistance.

MATERIALS AND METHODS

A lab work experiment and survey were conducted among patients who attended Zanko Medical Lab in Kalar City from August 2022 to March 2024. Patients of all ages and sexes were included, and samples were cultured using a manual routine protocol. All samples were collected conveniently based on the criteria for each sample type. Over 1500 samples were collected, 691 of which have been grown as significant colonies.

Sample Collection

Urine samples (20 mL) were obtained from patients using clean-catch midstream methods in sterile universal containers that have secure lids. All samples were received during 2–8 pm, using the convenience method, since the opening period of the medical clinics in the city. Additional clinical samples, such as high vaginal swabs (HVSs), sputum, and various other specimens, were collected using sterile techniques to reduce contamination.

Bacterial Isolation and Identification

Common types of equipment were used for isolation, including the streaking method for growing bacteria and yeasts on different types of media plates, such as MacConkey and Blood agar, which were incubated at 35–37°C for 18–24 h. Bacterial colonies were then counted**;** significant colonies were defined as those with growth exceeding 10^5 CFU/mL in urine samples or those associated with clinical symptoms in sterile body sites, following Clinical and Laboratory Standards Institute guidelines.[2]

Identification Method

Isolates were subjected to species-level identification using the Vitek2 Compact system (bioMérieux, France). Before identification, microbial suspensions were adjusted to 0.5 McFarland standards using a DensiCheck system (bioMérieux, France), following the manufacturer's protocol.

Vitek 2 Kits

To provide a broader spectrum of diagnostic accuracy, Gram-negative and Gram-positive bacteria were differentiated using GN and GP ID kits, and yeast strains were also identified with YST kits. The advanced diagnostic capabilities of the Vitek2 system greatly expedited the accurate identification of common and rare pathogens, which is in accordance with the recent direction in clinical microbiology.[9]

RESULTS AND DISCUSSION

A total of 691 strains were isolated at different ages in the study, 66.6% (460/691) of isolates from female, while 33.4% (231/691) from male. There was a response rate of 72.7% (691/950) captured and an identification rate of 94.9% (656/691). All isolates were Gram-negative with 59.04% (408/691), Gram-positive with 34.15% (236/691), and yeasts with 1.75% (12/691), but other low discrimination rates with the Vitek 2 instrument of 5.07% (35/691), as shown in Table 1 and Figure 1.

The common species of Gram-negative bacteria identified in urine specimens were *E. coli*, *K. pneumonia*, and *P. aeruginosa*, as shown in Table 2. *E. coli* and *K. pneumonia* occurred more frequently in females, whereas *P. aeruginosa* was more prevalent in males [Figure 2]. *Staphylococcus haemolyticus*, *E. faecalis*, and *Streptococcus agalactiae* were the most common gram-positive isolates, predominantly identified from urine samples [Table 3].

Urine samples accounted for 84.2% (582/691) of isolates, followed by HVSs (7.9%), sputum (2.9%), and various other sample types. This focus highlights the urinary tract as the primary infection site in the study population. Furthermore, Figure 3 represents the percentage distribution of common individual isolated, as well as uncommon isolated in the city.

This study demonstrates a predominance of Gram-negative bacteria (59.04%) among the isolated strains, consistent with previous research in Iraq by Faraj and Maaroof,[10] which showed that 90% of isolates were common bacteria, 8% were rare pathogens, and 2% were unidentified or poorly characterized. Conversely, by Gharavi *et al*.[2] found that *E. coli* constituted 72.16% of uropathogens, followed by *K. pneumoniae* (10.3%) and *S. agalactiae* (5.7%). Additional studies by Romaní *et al*. and Rajivgandhi *et al*.[6,8] further underscored *E. coli* as the most commonly isolated pathogen in UTIs. Thari *et al*.[11] reported a higher prevalence of *E. coli* in females (49.05%) compared to males (28.30%).

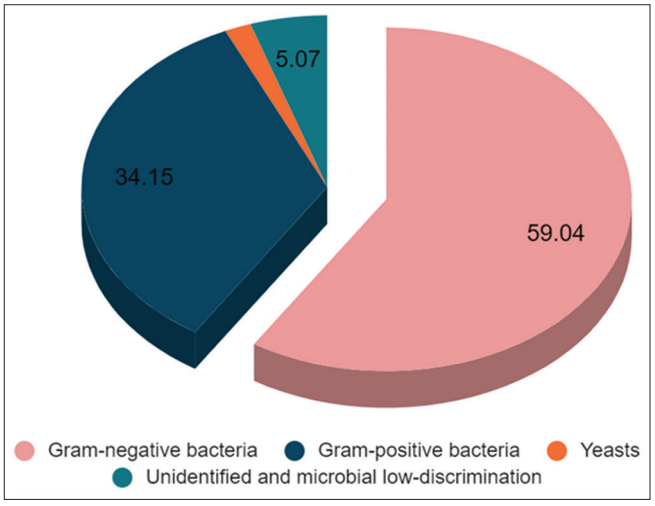

Figure 1: The frequency of microbial groups

Table 1: The overall distribution of microbial groups

Microbial name	Frequency	%	Patient sex		Samples			
			Female	Male	Urine	HVS	Sputum	Others
Gram-negative bacteria	408	59.04	259	149	360	18	14	16
Gram-positive bacteria	236	34.15	166	70	192	25	3	16
Yeasts	12	1.74	9	3	6	6	0	0
Unidentified and microbial low-discrimination	35	5.07	26	9	24	6	3	2
Total	691		460	231	582	55	20	34

HVS: High vaginal swab

Table 2: Distribution of Gram-negative bacteria according to the sex and type of samples

Microbial name	Frequency	Patient gender		Samples			
		Female	Male	Urine	HVS	Sputum	Others
Escherichia coli	267	191	76	248	15	0	4
Klebseilla pneumoniae	54	43	11	43	3	6	2
Pseudomonas aeruginosa	51	6	45	45	0	0	6
Proteus mirabilis	6	5	1	6	0	0	0
Morganella morganii	3	2	1	3	0	0	0
Burkholderia cepacia	3	0	3	3	0	0	0
Sphingomonas paucimobilis	3	2	1	2	-	1	-
Acinetobacter xylosoxidans	2	1	1	1	0	0	1
Serratia marcenscens	2	1	1	1	0	0	1
Stenotrophomonas maltophilia	2	1	1	1	0	1	0
Ochrobactrum anthropi	2	1	1	2	0	0	0
Enterobacter aerugenes	2	1	1	2	0	0	0
Escherichia vulneris	1	-	1	-	-	1	-
Klebsiella oxytoca	1	-	1	-	-	-	1
Proteus vulgaris	1	-	1	-	-	-	1
Enterobacter cloacae	1	-	1	1	-	-	-
Citrobacter freundii	1	-	1	1	-	-	-
Acinetobacter baumannii	1	1	-	-	-	1	-
Acinetobacter lwoffii	1	-	1	-	-	1	-
Aeromonas veronii	1	1	-	-	-	1	-
Pseudomonas fluorescens	1	1	-	-	-	1	-
Aeromonas sobria	1	1	-	-	-	1	-
Lelliottia amnigena	1	1	-	1	-	-	-
Total	408	259	149	360	18	14	16

Those are outlined in gray: considered as a rare frequency. HVS: High vaginal swab

The frequent isolation of *P. aeruginosa* in males undergoing endoscopic surgeries aligns with Haji *et al*.,[7] highlighting its link to hospital-acquired infections in surgical patients. *Staphylococcus hemolyticus* was mainly found in sexually active females, as it colonizes mucosal and skin surfaces, increasing UTI risk following disruption of natural microbiota.[12,13] *Enterococcus faecalis* may be found in urine underscoring its importance in UTI causation and biofilm formation.[8,14] A woman's *Streptococcus agalactiae* is of great consequence in pregnancy, and its screening is needed to mitigate risks of preterm birth and neonatal infection.[5] Haji *et al*. isolate *Staphylococcus aureus* in urine samples, which suggests possible hematogenous spread or colonization from the perineum.[7]

S. saprophyticus is highly prevalent among younger, sexually active females. Emphasize its role in uncomplicated UTIs due to its strong adherence to uroepithelial cells and its evasion of host defenses.[2] The diversity of species, which are not frequently isolated like *Aeromonas* spp. is in accordance with the observations made by Rajivgandhi *et al*. and Human Microbiome Project Consortium.[3,15] regarding the composition

Table 3: Distribution of gram positive bacteria according to the sex and type of samples

Microbial name	Frequency	Patient gender		Samples			
		Female	Male	Urine	HVS	Sputum	Others
Staphylococcus hemolyticus	82	51	31	74	7	1	0
Enterococcus faecalis	59	47	12	52	5	0	2
Streptococcus agalactiae	18	12	6	13	1	0	4
Staphylococcus aureus	14	7	7	6	1	0	7
Staphylococcus saprophyticus	10	10	0	9	1	0	0
Staphylococcus epidermidis	8	5	3	6	1	0	1
Streptococcus spp.	8	5	3	8	0	0	0
Staphylococcus hominis	4	3	1	3	0	1	0
Kocuria kristinae	4	3	1	2	2	0	0
Streptococcus pneumoniae	3	1	2	2	0	1	0
Lactococcus garvieae	3	3	0	1	2	0	0
Streptococcus pseudoporcinus	2	1	1	2	0	0	0
Granulicatella elegans	2	1	1	1	0	0	1
Streptococcus gallolyticus spp	2	2	0	2	-	-	-
Staphylococcus warneri	2	2	0	2	0	0	0
Streptococcus intermedius	1	1	-	-	1	-	-
Streptococcus thoraltensis	1	1	-	-	1	-	-
Streptococcus pyogenes	1	1	-	1	-	-	-
Streptococcus sanguinis	1	1	-	1	-	-	-
Streptococcus mutans	1	1	-	1	-	-	-
Streptococcus mitis/oralis	1	1	-	1	-	-	-
Streptococcus parasanguinis	1	1	-	1	-	-	-
Enterococcus avium	1	-	1	1	-	-	-
Streptococcus iniae	1	1	-	-	1	-	-
Streptococcus alactolyticus	1	1	-	1	-	-	-
Staphylococcus lowdeni	1	1	-	-	1	-	-
Staphylococcus kloosii	1	1	-	-	1	-	-
Staphylococcus lentus	1	1	-	1	-	-	-
Staphylococcus sciuri	1	1	-	1	-	-	-
Kocuria rosea	1	-	1	-	-	-	1
Total	236	166	70	192	25	3	16

Those are outlined in gray: considered as a rare frequency. HVS: High vaginal swab

of microbial communities being complicated. Variations in pathogen prevalence in different studies could have stemmed from geographic variations in population, health care, and/or research approaches.

The results from this study are very crucial when treating infections and limiting their spread. There is a high number of species like *E. coli* or *K.pneumoniae* among Gram-negative bacteria, which means we need potent first-line antibiotics targeted toward these microorganisms.[2,8] Also, according to Chaban *et al*. and Kim *et al*.,[5,16] females suffer from abdominal UTIs more than males. This is due to the female's organ structure and function. Therefore, there is a need to devise the diagnosing and treatment methods that cater to the differential.

The frequent occurrence of *P. aeruginosa* in males who are undergoing endoscopic procedures signifies the need for improvement of infection control practices during the surgery. This is further extended by Romaní *et al*. and Haji *et al*.[6,7] The identification of less frequently detected *Aeromonas* spp. and *Stenotrophomonas maltophilia* also calls for the use of better diagnostic methods targeting rare infections.[15,17] Changes in the above mentioned factors in the healthcare system would presumably contribute to decreasing antibiotic resistance and better the health of patients.

The finding of more unusual pathogens such as *Granulicatella elegans*, and *Acinetobacter* spp. underscores the need for improved diagnostic tools to detect under-appreciated pathogens,[3,15] Some of these, such as *Aeromonas*

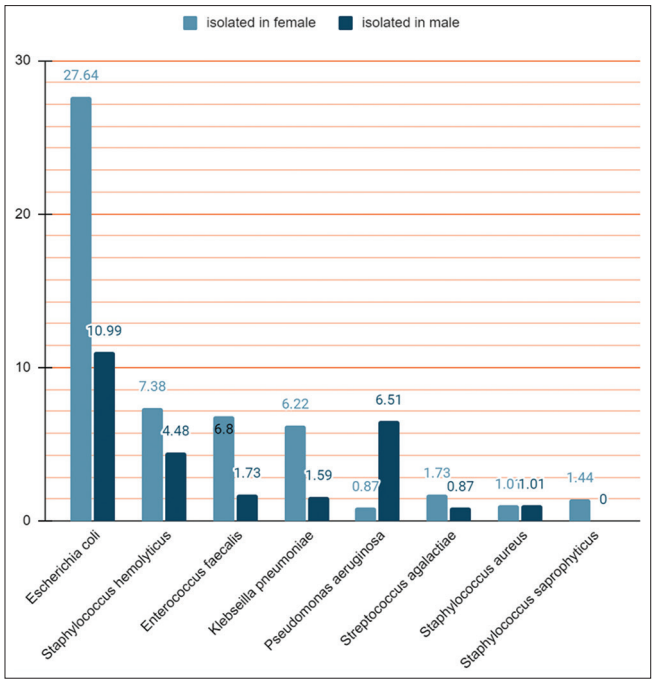

Figure 2: The percentages of common bacterial strains isolated in males and females

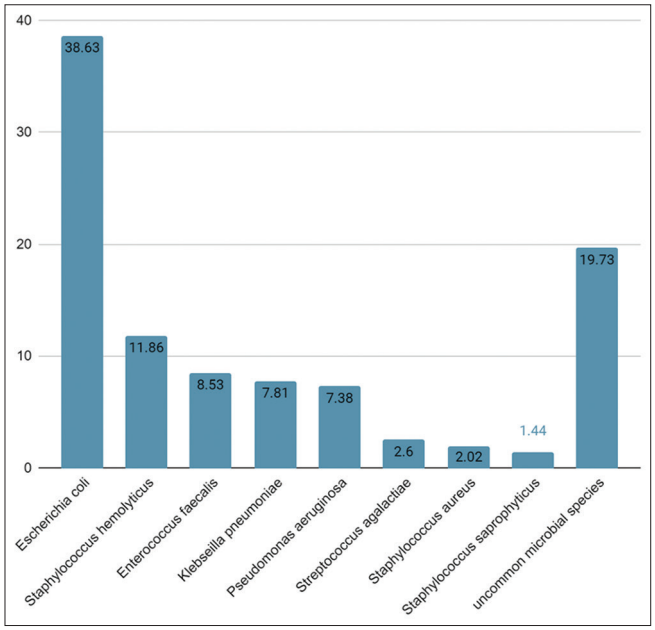

Figure 3: Common bacterial species isolated in Kalar city

sobria and *Lelliottia amnigena*, are being isolated for the first time in the study city, including both Gram-negative and Gram-positive bacteria, depending on the previous study by Faraj and Maarof.[18]

The results are consistent with Chaban *et al*.[5] what found, as there seem to be more pronounced differences in microbial communities across body sites and different body metamorphic phases. Some rare strains of bacteria, such as *Aeromonas veronii* and *S. maltophilia* substantiate the importance of microbial knowledge in the etiological diagnosis of infection and its management. This has also been researched by Van Camp *et al*. and Barbossa *et al*.[3,17] which further highlights this assertion. These findings suggest the need for better diagnostic systems that perform better in detecting the broad range of important clinical microbes for more effective treatment outcomes.

The study indicated that the VITEK 2 Compact system has the highest yield in the identification of microorganisms at an efficiency of 94.9%. Hence, this now serves as a primary apparatus in clinical microbiology. A lot of common as well as uncommon types of microorganisms can be identified by it. Romaní *et al*. and Haji *et al*., whose research demonstrated that the device exceeds older ones in terms of accuracy and reliability.

The new technology and automation of tools such as the VITEK 2 Compact permit the detection of some rare bacteria, including *Burkholderia cepacia* and *Morganella morganii*. This bolsters the work conducted by Human Microbiome Project Consortium and Barbossa *et al*.[15,17] who first demonstrated that these systems could be effectively automated for the detection of hundreds of species.

One limitation of the study is the narrow geographical area, as this would make it difficult to extrapolate the results to different areas that might have distinct microflora. Furthermore, the urine and the vaginal swabs, which are the specific sample types used, might represent significant variations in comparison to what is present in other clinical samples, as proposed.[3,6] Finally, the study was descriptive in nature, and no inferential statistical comparisons were made across groups, limiting the ability to draw causative conclusions.

CONCLUSION

The research indicates that specialized diagnostic microbiology is nearly the same as split diagnostic in the clinical laboratory. The system tool facilitated efficient and broad-spectrum microbial identification at a high rate. However, since no comparisons were made with manual or molecular techniques, no definitive conclusions regarding the superiority of the system can be drawn. Some isolates remained unidentified or had low discrimination, emphasizing the need for supplementary molecular techniques such as PCR and sequencing in future work.

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