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Original Research Article

Antibiogram and Bacterial Profile Analysis of Various Clinical Samples at Tertiary Healthcare Centre in North-West region of Punjab

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ABSTRACT

Background: Antimicrobial resistance is a significant barrier in achieving the sustainable development and is one of the leading global public health menaces to humanity. Present study determined the antibiogram profiles of bacteria isolated from different body site infections among patients admitted to tertiary care Hospital, Punjab, Northwest India.
Methods: A cross-sectional study was carried out using 11201 clinical samples at the tertiary care hospital Patiala from June 2023 to June 2024. Following the criteria of the Clinical and Laboratory Standards Institute *S. aureus* isolates and further tested for antimicrobial activity using standard procedures
Results: From the 11201 clinical specimens processed, a total of 1638 urine, 403 blood and 2126 other body fluids (pus and sputum) were culture positive for bacterial pathogens. The prevalence of infection was highest among middle aged males in blood and other fluids where middle aged female's predominance was found in urine infection. In urine samples, *Escherichia coli* (63.67%) from Gram-negative and *Enterococcus* (6.53%) and *S. Aureus* (4.45%) from gram positive were the predominant bacterial. Blood sample showed the prevalence of *K. pneumonia* (28.28%) among gram negative and *Enterococcus* (27.79%) among gram positive. *K. pneumonia* (20.93%) and *S. Aureus* (29.25%) were predominant in other fluid infections. antimicrobial resistance pattern in all clinical samples and results suggest that most of the bacteria isolated from studied clinical samples are less susceptible ranging from 0% to 35% mostly towards penicillin, norfloxacin, Cefuroxime and ampicillin. Intermediate susceptibility ranged from 40%-85% towards amikacin, Gentamicin and Meropenem. High susceptibility varied according to bacteria and sample type and it ranged between 90%-100%. In urine sample high susceptibility was observed for *P. aeruginosa* (100%) and *A. Baumanni* (100%). In Blood samples *S. aureus* showed the highest susceptibility towards amikacin (100%). Many antibiotics showed 100% susceptibility in other body fluids infections such as *P. aeruginosa* towards Norfloxacin, *S. aureus* towards amoxicillin, *enetrococcus* towards ampicillin, gentamicin and amikacin.
Conclusion: Overall result of the present investigation showed that penicillins are least susceptible and amikacin is highly susceptible antibiotic against the studied infections. By

focusing on locally prevalent antibiotic resistance patterns instead of regionally and internationally available data on antibiogram, we may ensure faster patient recovery at lower costs and avoid the unwarranted development of drug resistance.

Keywords: Antibiogram, Antibiotic resistance, Bacterial isolates, clinical specimen, Punja

INTRODUCTION

Antimicrobial resistance is a significant barrier in achieving the sustainable development and is one of the leading global public health menaces to humanity. Over the last two decades, antibiotic resistance has skyrocketed, and the primary cause of this can be irrational use, and the accessibility of these medications over-the-counter. [1] Microbial infections are responsible for at least 25% of the 60 million fatalities that occur worldwide each year.

Clinical infections such as bloodstream, UTIs and body fluid infections continue to be major causes of morbidity and mortality among hospitalized patients. [2] Bloodstream infections caused by bacteria or other germs are referred to as bloodstream infections (BSIs), septicemia, or bacteremia. Many pathogens, including as bacteria, viruses, and fungi, can cause bloodstream infections (BSIs). 7. *Staphylococcus*, *Escherichia*, and *Klebsiella* are the three most prevalent bacterial genera that cause bloodstream infections. [3] Urinary tract infection (UTI) is another most frequent hospital-acquired infection and the second most frequent reason for hospital visits. Gram positive organisms including *Staphylococcus saprophyticus*, *Staphylococcus aureus*, and *Enterococci*, as well as Gram negative enteric bacilli like *Escherichia coli (E. coli), Klebsiella spp.*, and *Proteus spp*., are the most common causes of UTIs. [4] Lastly, bacterial infections of bodily fluids can result in invasive conditions like bacterial meningitis, bacteremia, sepsis, bacterial peritonitis, and other problems. [5]

Drug resistance is a significant issue in low-income nations due to the high rate of illnesses, the inappropriate use of antibiotics, the easy availability of medications, and the lack of access to antimicrobial susceptibility testing. [6] The Indian populace is recognized as the world's largest consumer of antibiotics worsening AMR situation in India thus an action plan for its control is considered crucial. [7]

The region-wide report of culture and sensitivity data is provided by hospital antibiogram which indicates how an organism's susceptibility pattern is evolving. [8] Consequently, it will assist clinicians in selecting the right antibiotic, reducing the inappropriate use of antibiotics and the emergence of resistance. Thus, the purpose of this study is to ascertain the antibiotic susceptibility pattern and bacteriological profile of UTI bacterial isolates from a tertiary healthcare facility.

MATERIALS & METHODS

From June 2023 to June 2024, a prospective cross-sectional investigation was carried out at the tertiary care hospital, Punjab. The current investigation comprises 11201 isolates from different clinical specimens. Ethical clearance from the Research and Ethical committee was taken.

This study includes all the clinical samples that were properly collected, labeled, properly transported and processed for aerobic bacterial cultures. Isolates obtained from blood, urine, pus, and sputum, were included in this study. According to standard microbiological procedures received samples were processed.

Using the conventional Kirby Bauer disk diffusion technique, all isolates' antimicrobial susceptibility was assessed in accordance with Clinical Laboratory Standards Institute (CSLI) guidelines. Antibiotics included were amoxicillin (20mcg), ampicillin (50mcg), gentamicin (30mcg), amikacin (15mcg), cefoxitin (30mcg), clindamycin (20mcg), doxycycline (50mcg), erythromycin (15mcg).

RESULT

The results show the distribution of several bacterial species that were isolated from clinical samples in the North West India during 2023. A total of 5053 urine samples, 2127 Blood samples and 4021 other body fluids such as pus, sputum samples were studied. Out of which 1638 (32.41%) urine isolates, 403 (18.94%) blood isolates and 2126 (52.87%) other body fluids isolates were reported in Table 1.

Among gram negative urine isolates *Esch.coli* (63.67%) is the most common bacterial isolates found followed by *K. pneumonia* (17.52%), *P. aeruginosa* (2.62%), *ABComplex* (1.28%), *Citrobacter* (1.09%), *Proteus* (0.97%), *Enterobacter* (0.79%), *while enterococcus* (6.53%) is most prevalent among gram positive urine isolates followed by *S. aureus* (4.45%) and *MRSA* (1.03%).

In case of Blood isolates Gram negative show different trend with *K. pneumonia* (28.28%) highest prevalent bacteria followed by *ABComplex* (8.68%), *Esch. coli* (7.94%), *P. aeruginosa* (5.45%), *Citrobacter* (2.72%), *Enterobacter spp* (1.98%) *and Proteus spp* (1.24%). Gram positive blood isolates showed similar trend as urine isolates with high prevalence of *Enterococcus* (27.79%) afterward *S. aureus* (13.39%) and *MRSA* (2.48%).

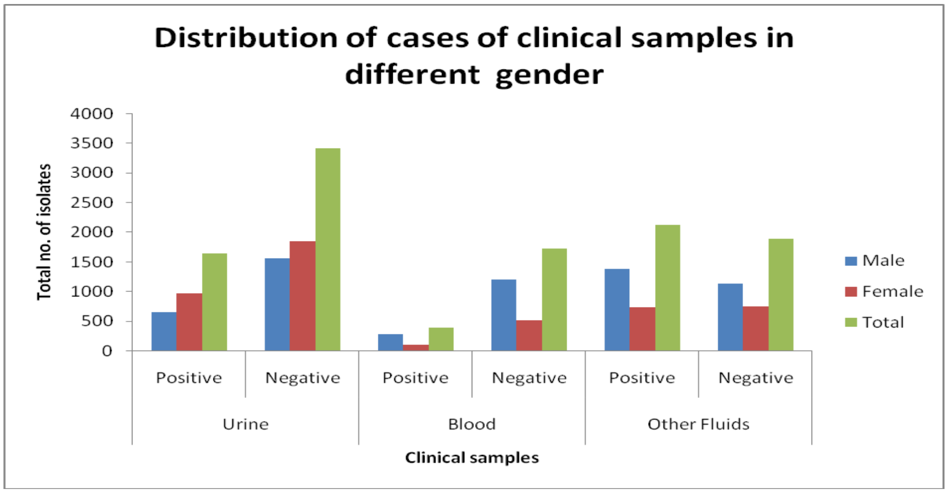
Other body fluids such as pus and sputum showed highest number of positive isolated among all samples with highest prevalence of *S.aureus* (29.25%) in gram positive bacteria *and K. pneumonia* (20.93%) in gram negative bacteria. After *K. pneumonia* among gram negative prevalence of *ABComplex* (15.75%) was observed followed by *Esch.coli* (11.52%), *P. aeruginosa* (3.80%), *Proteus* (0.65%), *Citrobacter* (0.61%), *Enterobacter* (0.42%) and among gram positive after *S.aureus* prevalence of *enterococcus* (15.85%) and *MRSA* (1.17%) was observed.

Table 1: Distribution of various isolates among clinical samples.

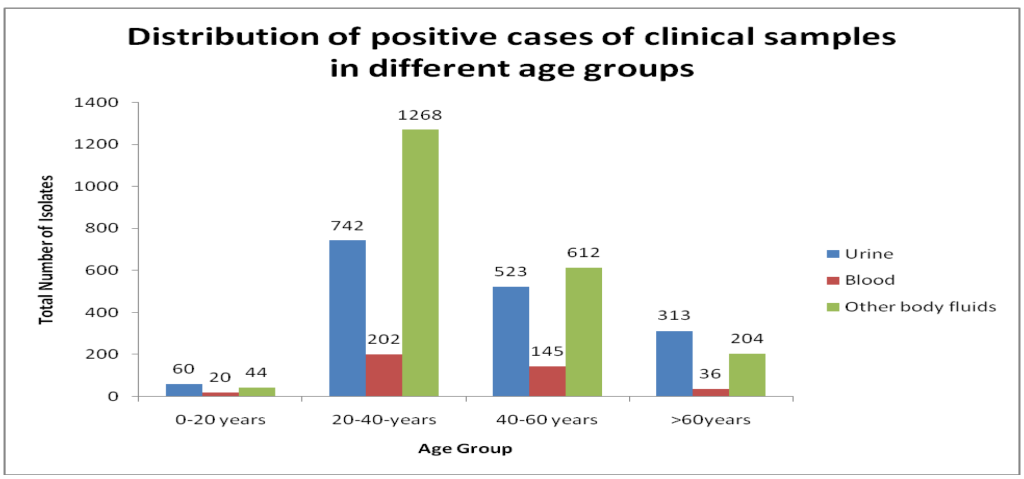
Organism	Gram staining	URINE SAMPLES	BLOOD SAMPLES	OTHER BODY FLUIDS
Esch.coli	Gram negative microorganism	1043	32	245
Klebsiella Pneumonia	Gram negative microorganism	287	114	445
Pseudomonas Aeruginosa	Gram negative microorganism	43	22	81
Acinetobacter Baumanni Complex	Gram negative microorganism	21	35	335
Citrobacter Species	Gram negative microorganism	18	11	13
Proteus Spp	Gram negative microorganism	16	5	14
Enterobacter Spp	Gram negative microorganism	13	8	9
Enterococcus Spp	Gram positive microorganism	107	112	337
Staphylococcus Aureus	Gram positive microorganism	73	54	622
MRSA	Gram positive microorganism	17	10	25
Total		1638	403	2126

Among the studied population, it was observed that female patients 979 (59.77%) showed high prevalence than male patients 659 (40.23%) in urine infection while in other clinical samples males [291(72.20%); 1382 (65%)] were dominating than females [112(27.80%); 744(35%)] both in blood and other body fluids as shown in figure 2.

Table 2: Gender distribution of all clinical samples.



Age group distribution of all clinical samples showed the similar trend. It was found that in all samples mostly 20-40 years age range is more infected followed by 40-60years, >60years and 0-20 years as depicted in the figure 3.



Antibiogram of urine isolates showed that gram negative *Esch. coli* bacteria have lowered susceptible towards commonly used antibiotics amoxicillin/penicillin (28.3%), norfloxacin (29.4%), Ceftazidime (13.2%) and intermediate susceptibility towards amnioglycosides (61-68%), carbapenems (77.3%) and nitrofurantoin (65.9%). *K.pneumoniae* had lower susceptibility towards amoxicillin/penicillin (33.7%), Ceftazidime (22.7%), nitrofurantoin (25.5%) while intermediate susceptibility is seen against Aminoglycoside (51%-64%), carbapenems (54.8%), complete susceptibility towards norfloxacin (100%). *P. aeruginosa and ABComplex* is completely susceptible towards Ceftazidime (100%) *ABComplex* have complete susceptibility against Gentamicin (100%) also. *P. aeruginosa* showed Intermediate susceptibility towards Gentamicin (77.8%), amikacin (87%), carbapenems (90.9%). *P. aeruginosa and ABComplex both showed* resistance towards amoxicillin/penicillin (5.9%; 16.7%) and nitrofurantoin (13.3%; 37.5%).

Among Gram positive *Enterococcus* had lower susceptibility macrolides (33.3%), clindamycin (4%) and doxycycline (33.3%).

Intermediate susceptibility against ampicillin (60%), amikacin (42.9%) and nitrofurantoin (89.3%). Intermediate susceptibility of *S. aureus* was seen against nitrofurantoin (95.7%), amnioglycosides (90.5%; 80%), clindamycin (70%) and doxycycline (63.2%).

Percentage of Susceptible Isolates in Urine Samples

GROUP	ORGANISMS	Penicillin's		Amino-glycosides		Macrolides	Quinolones		Cephalosporins				Carbapenems	Miscellaneous			
		Amoxicillin/ Clavulanic acid	Ampicillin	Gentamicin	Amikacin	Azithromycin	Norfloxacin	Ofloxacin	Cefuroxime	Ceftazidime	Cepnoperazone	Cefoxitin	Meropenem	Clindamycin	Doxycycline	Nitrofurantoin	Tetracycline
Gram Negative	*Escherichia coli*	28.3		61.6	68.8		29.4			13.2			77.3			65.9	
	Klebsiella pneumoniae	33.7		64.3	51.5		100			22.7			54.8			25.5	
	Pseudomonas aeruginosa	5.9		77.8	87					100			90.9			13.3	
	Acinetobacter baumannii	16.7		100	58.3					100			64.3			37.5	
Gram positive	*Enterococcus faecalis*		60		42.9	33.3								4	33.3	89.3	
	Staphylococcus aureus		32.1	90.5	80							21.4		70	63.2	95.7	

Susceptibility profile of blood samples has shown that most of the gram negative bacteria (*K. pneumoniae*, *ABC-Complex*, *Esch. coli*, *P. aeruginosa*) are less susceptible to many antibiotics such as Penicillin, Amikacin, norfloxacin and Cefuroxime which ranges from (0% to 30%). Gram positive has varied range of susceptibility from complete, intermediate to low. Complete susceptibility of *S. aureus* was observed for amikacin. Intermediate susceptibility of *S. aureus* was seen for Gentamicin (91.2%), doxycycline (80%) and clindamycin (63.2%) while low susceptibility was observed against penicillin (33.3%) and cefoxitin (22.2%). *Enterococcus* susceptibility profile showed that it has low susceptibility towards clindamycin (15.8%) and amikacin (37.5%), Intermediate towards ampicillin (47.4%) and doxycycline (57.9%). However, it has high susceptibility to Gentamicin (71.4%).

Percentage of Susceptible Isolates in Blood Samples																	
		Penicillin's		**Amino-glycosides**		**Macrolides**	**Quinolones**		**Cephalosporins**				**Carbapenems**	**Miscellaneous**		
GROUP	**ORGANISMS**	**Amoxicillin/ Clavulanic acid**	**Ampicillin**	**Gentamicin**	**Amikacin**	**Azithromycin**	**Norfloxacin**	**Ofloxacin**	**Cefuroxime**	**Ceftazidime**	**Cephoperazone**	**Cefoxitin**	**Meropenem**	**Clindamycin**	**Doxycycline**	**Tetracycline**
Gram Negative	*Klebsiella pneumoniae*	23.2			32.1				20.2							
	Acinetobacter baumannii	33.3			54.5				40.0							
	Escherichia coli	31.6			70.0		0		22.2							
	Pseudomonas aeruginosa				42.9											
Gram positive	*Staphylococcus aureus*	33.3	44.2	91.2	100							22.2		63.2	80.0	
	Enterococcus faecalis		47.4	71.4	37.5									15.8	57.9	

Antibiogram of other body fluids showed all isolated gram negative bacteria (*K. pneumoniae*, *ABC-Complex*, *Esch. coli*, *P. aeruginosa*) are less susceptible to intermediate most of the antibiotic such as penicillin, Cefuroxime and norfloxacin. Low susceptibility ranges form (0%-30%) while intermediate susceptibility ranged from (40% - 66.7%). However, *P. aeruginosa* showed complete susceptibility towards norfloxacin (100%). Trend of gram positive bacteria showed that most are completely susceptibility (100%) towards amoxicillin, ampicillin, Gentamicin, amikacin. Intermediate susceptibility was observed in *S.aureus* towards Gentamicin (96%), amikacin (92.3%), doxycycline (78.4%), clindamycin (75.8%) and cefoxitin (41.7%). *Enterococcus* showed complete resistance towards clindamycin. And intermediate towards doxycycline (50%).

Percentage of Susceptible Isolates in Other Body Fluids

GROUP	ORGANISMS	Penicillin's		Amino-glycosides		Macrolides	Quinolones		Cephalosporins				Carbapenems	Miscellaneous		
		Amoxicillin/ Clavulanic acid	Ampicillin	Gentamicin	Amikacin	Azithromycin	Norfloxacin	Ofloxacin	Cefuroxime	Ceftazidime	Cephoperazone	Cefoxitin	Meropenem	Clindamycin	Doxycycline	Tetracycline
Gram Negative	*Klebsiella pneumoniae*	27.9			57.8		4.4		27.9							
	Acinetobacter baumannii	40			77.8				20							
	Escherichia coli	19.7			66.7		0		5.7							
	Pseudomonas aeruginosa	6.9			83.3		100		0							
Gram positive	*Staphylococcus aureus*	100	24.6	96	92.3							41.7		75.8	78.4	
	Enterococcus faecalis		100	100	100									0	50	

DISCUSSION

In the current investigation, bacterial isolates obtained from various samples such as urine, blood and other body fluids (pus and sputum) were analyzed to recognize their antibiotic susceptibility patterns in Northwest India. Our findings for urine isolates, are consistent with prior research, which consistently identifies Escherichia coli as the predominant pathogen responsible for urinary tract infections (UTIs) in both hospital settings and the general population. [4] In a study conducted in eastern Nepal also reported a high incidence of *Esch. coli* (53.57%), *Klebsiella spp*(14.29%) and *Enterococcus* (11.90%). [9] Moreover, a study conducted in southern India found similar results, with *Esch. coli* being the most prevalent pathogen causing UTIs, followed by *Klebsiella pneumoniae* and *Enterococcus species.* [10] In North India, a study conducted in Delhi reported Escherichia coli as the predominant pathogen causing UTIs, followed by *Klebsiella pneumoniae* and *Enterococcus species.* [11] Similarly, a study in Punjab found *Esch. coli* to be the most common pathogen associated with UTIs, corroborating our findings. [12]

Blood isolates also showed are consistent with the previous findings. [13] An interesting study conducted in Gana on blood samples reported the high prevalence of *K. pneumonia* and *Esch. coli* as least dominating among gram negative, as found in the current study. [14] Gram positive profile of bacterial isolates also showed the similar pattern as found in previous study from U.S.A where the author found the higher dominance of *S. aureus* (211) and *enterococcus* (153). [15] However a study from North India showed that among gram negative bacteria, the predominance of *Acinetobacter species* followed by *K. pneumonia* was observed and among gram negative *S. aureus* was leading bacterial infection. [16]

In the present study other body fluids (pus and sputum) showed the highest prevalence of *S. aureus* in gram positive and *K. pneumonia* in gram negative bacteria. This finding is similar to study conducted in Nepal where author also found the highest prevalence of *S. aureus* among gram positive and *K. pneumonia* among gram negative bacteria. [17] A study published in Lancet showed 5 year data found the predominance of *S. aureus*, *K. pneumonia* and *pseudomonas.* [18] Predominance of *S. aureus* reflects its role in soft tissue infections and its ability to form biofilm, which contributes to its persistence and pathogenicity.

According to the current study, female patients have higher prevalence of infection rate than males align with prior published literature indicating the anatomical and physiological differences. Factors such as shorter urethra, anus proximity and hormonal fluctuations all can contribute to increased susceptibility in females and facilitate bacterial colonization. [19] The data from Mumbai, where a significant percentage of middle-aged female patients were diagnosed with bacterial illnesses in comparison to their male counterparts, are consistent with this. [20] A study from Aligarh University revealed similar trends. [21] Furthermore, studies carried out in other areas have also documented comparable patterns in the frequency of *Esch. coli* infections by gender. According to a research done in Karachi, Pakistan, middle-aged women were more likely than males to get urinary tract infections brought on by *Esch. coli.* [22] Similarly, a research in Sri Lanka found that females, especially those in their middle years, had a greater frequency of urinary tract infections caused by *Esch. coli.* [23] Together, these findings support the gender-specific variations in urinary tract *Esch. coli* infection prevalence that has been noted, especially among middle-aged women, and emphasize the necessity of focused therapies for this population. In contrast, blood and other body fluids showed the high prevalence of male patients. The findings of the current study align with the findings of the previous published studies, a study done in North

Iran on blood culture showed the higher prevalence of male (116) than females. [24] In many cases, blood stream infection and fluids infections are common in males due to range of factors such as males are at high risk of endocarditic, intravenous drug use and innate immune response. [25]
Present study observed wide range of antimicrobial resistance pattern in all clinical samples and results suggest that most of the bacteria isolated from studied clinical samples are less susceptible ranging from 0% to 35% mostly towards penicillin, norfloxacin, Cefuroxime and ampicillin. Intermediate susceptibility ranged from 40%-85% towards amikacin, Gentamicin and Meropenem. High susceptibility varied according to bacteria and sample type and it ranged between 90%-100%. In urine sample high susceptibility was observed for *P. aeruginosa* (100%) and *A. Baumanni* (100%). In Blood samples *S. aureus* showed the highest susceptibility towards amikacin (100%). Many antibiotics showed 100% susceptibility in other body fluids infections such as *P. aeruginosa* towards Norfloxacin, *S. aureus* towards amoxicillin, *enetrococcus* towards ampicillin, gentamicin and amikacin. Overall result of the present investigation showed that penicillins are least susceptible and amikacin is highly susceptible. The findings of the present study align with broader pattern observed in the previous studies on antimicrobial resistance highlighting the worrisome trends of resistance mainly towards commonly used antibiotics like penicillin, norfloxacin, Cefuroxime and ampicillin. A study by Khameneh et al. 2016 indicated that common antibiotics particularly penicillin face increased resistance in both community and healthcare associated infections. [26] A study conducted in Pondicherry, South India, highlighted the prevalence of multidrug-resistant *Escherichia coli* isolates to conventional antibiotics like Ampicillin, Ciprofloxacin, and Amoxicillin-clavulanate. [27] Previous research highlighting the same tale as described in the current study about antimicrobial resistance pattern. [28] A study by Kapoor et al. demonstrated high rates of resistance among Gram-negative bacteria, including *Esch. coli* and *Klebsiella pneumoniae*, to commonly prescribed antibiotics such as Ampicillin, Ciprofloxacin, and third-generation cephalosporins. [29] Among gram positive organism, the present study has corroborated with global trends, demonstrating moderate to high sensitivity of Enterococcus spp. to various antibiotics, including Ciprofloxacin, Levofloxacin, and Nitrofurantoin. [30]

CONCLUSION

Overall result of the present investigation showed that *Escherichia coli* from Gram-negative and *enterococcus* and *S. Aureus* from gram positive were the predominant bacterial in urine infection. Blood sample showed the prevalence of *K. pneumonia* among gram negative and *enterococcus* among gram positive. *K. pneumonia* and *S. Aureus* were predominant in other fluid infections. Penicillins are least susceptible and amikacin is highly susceptible antibiotic against the studied infections. By focusing on locally prevalent antibiotic resistance patterns instead of regionally and internationally available data on antibiograms, we may ensure faster patient recovery at lower costs and avoid the unwarranted development of drug resistance.

Declaration by Authors

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