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Antimicrobial Resistance Patterns among Bacterial Pathogens Isolated from Clinical Specimens in Sheikh Zaid Hospital, Lahore

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Abstract:
This retrospective study was conducted to determine antimicrobial resistance patterns of bacterial pathogens among patients suffering from Urinary Tract Infections (UTI's) at the Pathology laboratory, Sheikh Zaid Hospital, Lahore. A total of 50 samples were collected from the different sources which include pus (n=24), followed by blood (n=14), and urine (n=12). Isolation and characterization of bacterial strains was done by conventional cultural, and biochemical methods for microbial enumeration. Antimicrobial susceptibility of bacterial isolates against penicillin, cefotaxime, tetracycline, augmentin, ciprofloxacin, and imipenem was tested by the disk diffusion method. The results revealed a higher prevalence of UTI's among females (71%) than males (36%). Prevalence of bacterial isolates was *Enterococcus faecalis* (42%), followed by *Staphylococcus aureus* (40%), and *Streptococcus pneumoniae* (18%). Among the six antibiotics tested, bacterial isolates were more resistant to Penicillin; with *S.pneumoniae* (100%), *E.faecalis* (98%), and *S.aureus* (85%). The majority of the bacterial isolates were resistant to penicillin that is mostly prescribed antibiotic and illustrated that more consumption of a specific antibiotic leads to the sustainability of resistance against those antibiotics.

Keywords: Urinary tract infections, bacterial pathogens, antimicrobial susceptibility, penicillin.

INTRODUCTION

Resistance of bacteria to antibiotics is a common phenomenon (Iqbal and Ashraf, 2018; Shawish *et al.*, 2020; Yunus *et al.*, 2016). Antimicrobial resistance (AMR) is the tendency of a microorganism to withstand the consequences of treatment that once could work to treat the microorganism. The term Antibiotic resistance (AR or ABR) is a subset of antimicrobial resistance. It applies only to bacterial species that become resistant to antibiotics. It is difficult to treat resistant microbes; the only way is to treat them with alternative medication or high doses of antimicrobials. However, these ways are more expensive as well as toxic (Kiffer *et al.*, 2007) microorganisms resistant to multiple antibiotics are known as multidrug-resistant (MDR). Some are drug-resistant (TDR) or extensively drug-resistant and are known as Superbugs (Börjesson *et al.*, 2016).

Urinary tract infection (UTI) is the second most common infectious presentation in community practice (Zetola *et al.*, 2005). Urinary tract infections are often treated with different broad-spectrum antibiotics when one with a narrow spectrum of activity may be inappropriate because of concerns about infection with resistant organisms (Mori *et al.*, 2007).

Medicinal plants have been used to alleviate or even cure infectious diseases. The herbal medicines were widely used as home remedies in the early days (Morais-Braga *et al.*, 2012). Medicinal plants have certain unique phytochemicals which possess antibacterial activity (Hussain *et al.*, 2016; Iqbal *et al.*, 2019; Iqbal *et al.*, 2015; Iqbal and Ashraf, 2019; Mouffouk *et al.*, 2019; Shahzad *et al.*, 2017).

Antibiotic treatment is considered as the most important reason promoting the emergence, selection, and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine. Antibiotics are used in humans for the treatment and control of bacterial infections (Groth *et al.*, 2012). New antibiotics are introduced at a much lower rate, and due to this fact, antibiotic resistance is widely recognized as a major threat to public health (Butler *et al.*, 2006). The urgent need is emphasized to strengthen the microbiological and epidemiological capacities of health care workers internationally to prevent transmission of nosocomial infections and to prepare them to address the problem of the emergence of multiple drug resistance among various bacterial isolates (Martone, 1998).

The prevalence studies of the microorganisms are essential to identify the most pathogenic organisms and resistant strains that will help to limit the spread of resistant strains and effective use of therapeutic agents (Khan *et al.*, 2013). Because of multidrug-resistant plasmids that may be easily transmitted, ESBL producing organisms are often resistant to other classes of antibiotics. Hence, the most appropriate name would be “multidrug-resistant organisms”. Second, because of the lack of an obvious marker to indicate the presence of such enzymes, routine susceptibility testing may not detect the presence of ESBLs (Mahony *et al.*, 2011).

The high global use of antibiotics, the rapid spread of multidrug-resistant bacteria, and the lack of new, effective antibiotics have led to an imminent threat to health systems and global development (Ali *et al.*, 2016; Li and Webster, 2018; O'Connell *et al.*, 2013). This study aimed to determine antimicrobial resistance patterns of bacterial pathogens among patients suffering from Urinary Tract Infections (UTI’s) at the Pathology laboratory, Sheikh Zaid Hospital, Lahore.

MATERIALS AND METHODS

Sample collection

The study was approved by the institutional research committee and the anonymity of patients was protected. During the study period, a total of fifty clinical samples of urine (n=24), pus (n=14), and blood (n=12) from patients suffering from Urinary Tract Infections (UTI's) (Figure 1) were randomly collected in sterile bottles at Sheikh Zaid Hospital, Lahore. The date, time, and number of patients were labeled on the container and transported to the laboratory within 2 hours of collection (Chakraborty *et al.*, 2011; Saleem *et al.*, 2018a; Saleem *et al.*, 2018b). The samples were processed for microbiological examination at the Pathology laboratory, Sheikh Zaid Hospital, Lahore.

Fig. 1. Samples collected from UTI Patients. a): urine, b): pus and c): blood.

Primary culture

Blood agar, MacConkey agar, and Nutrient agar but mainly CLED agar media were prepared following the manufacturer's instructions; pH was adjusted, autoclaved, poured in sterilized Petri plates, and was incubated at 37 °C for 24 hours for sterility check. Only sterile agar plates were selected for primary culturing. Samples were centrifuged at 6000 rpm for 5 minutes after the sediments (pus) settled into the bottom of tubes and supernatant was discarded. Primarily sediments obtained by centrifugation of urine were cultured on Blood agar, MacConkey agar, Nutrient agar, and CLED agar by spread out technique. Then these culture plates were incubated at 37 °C for 24 hours (Saleem *et al.*,, 2018b).

Purification of Bacterial Isolates

Bacterial colonies having different morphology were selected for purification by multiple streaking. Then bacterial colonies with different morphological characteristics were picked by a loop from primary culture plates and cultured on Blood agar, MacConkey agar, and Nutrient agar plates. The pure cultured plates were labeled and incubated at 37 °C for 24 hours (Hussain *et al.*,, 2016).

Identification of bacterial isolates

All of the purified bacterial isolates (n=50) were identified based on colony morphology, microscopy, and biochemical tests following the standard protocols of Bergey's Manual of Determinative Bacteriology (Bergey and Holt, 1994; Iqbal *et al.*, 2016; Saleem *et al.*,, 2018b).

Antibiotic Sensitivity Testing

Antimicrobial susceptibility of isolates was tested for all bacterial Uropathogens by the disk diffusion method using Muller Hinton agar according to Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2016). The antibiotic discs were: Penicillin, Cefotaxime, Tetracycline, Augmentin, Ciprofloxacin, and Imipenem. The bacterial colonies were suspended with McFarland standard. Using a sterile inoculating loop picked the bacterial colony and dispensed it into the saline solution. Comparing the McFarland standard adjusted the turbidity of the suspension. Sterile swabs were dipped into the inoculums tubes and inoculated onto Muller Hinton agar plates. Antibiotic discs were placed on the surface of the inoculated agar plate. Plates were incubated at 37 °C for 24 hours. Examined the plates after 24 hours and measured the diameter of the zone of inhibition. Identify the sensitivity or resistance of the bacteria against all tested drugs by using the available CLSI guidelines.

Statistical analysis

A Chi-square test was applied to correlate and analyze the obtained data. Statistical analysis was carried out with the probability of less than 5% i.e. 0.05 level were considered as significant.

RESULTS

The results showed that among 50 samples collected, there was a higher prevalence of UTI's among females (71%) than males (36%).

Identification of bacterial isolates

All of the purified bacterial isolates (n=50) were identified based on culture characteristics, microscopic morphology, gram stain (Table 1), and biochemical profiles (Table 2).

Table 1. Microscopic and Colonial characteristics of bacterial isolates from clinical samples.

Bacterial species	**Colony characteristics**			**Morphological characteristics**		
	Color on agar	**Color on MacConkey agar**	**Color on blood agar**	**Gram staining**	**Motility test**	**Oxygen requirement test**
Staphylococcus	Uniform, opaque, and Deep yellow colonies	No growth to slight growth (pale pink)	Yellow to cream or white colonies	+ cocci	Non-motile	Facultative anaerobe
Streptococcus	Gray to whitish-gray surrounded by a weak zone of beta hemolysis	Pink colonies	Yellow-brown color	+ rods	Non-motile	Facultative anaerobic
Enterococcus	Red-colored compounds that give colonies a pink to red coloration	Tiny red colonies	Black complexes as a black zone	+ rods	Non-motile	Facultative anaerobe

Table 2. Biochemical identification of bacterial isolates from clinical samples.

Biochemical test		***Staphylococcus***	***Enterococcus***	***Streptococcus***
Gram staining		+	+	+
Motility test		–	–	–
Catalase test		+	–	+
Oxidase test		–	–	–
Indole production test		–	–	–
Methyl red test		+	–	–
Vogues Proskauer test		+	+	+
Lactose fermentation test		+	–	+
Mannitol salt agar		+	–	+
Citrate utilization test		+	+/–	+
Eosin methylene blue		–	–	–
Urease production test		–	–	–
Triple sugar iron test	Slant	K	K	K
	Butt	A	A	K
	Gas	–	–/=	–
	H_2S	–	+/–	–

Prevalence of bacteria

Out of biochemically identified bacterial isolates (n=50), the highest number was of *Enterococcus faecalis* (42%), followed by *Staphylococcus aureus* (40%), and *Streptococcus pneumoniae* (18%) (Table 3).

Antibiotic sensitivity testing

According to Antibiotic sensitivity testing results, *Staphylococcus aureus* was more resistant to Penicillin 85% followed by Cefotaxime 65%, Tetracycline 50%, Augmentin 45%, Ciprofloxacin 33%, and Imipenem 20%. *Streptococcus pneumoniae* was more resistant to Penicillin 98% followed by Cefotaxime 70%, Tetracycline 60%, Augmentin 55%, Ciprofloxacin 50%, and Imipenem 0%. *Enterococcus faecalis* was more resistant to Penicillin 100% followed by Cefotaxime 90%, Tetracycline 70%, Augmentin 24%, Ciprofloxacin 55%, and Imipenem 10% (Table 4).

Table 3. Prevalence of bacterial isolates identified by conventional biochemical characterization.

Name of bacteria isolated	Total number of samples	Number of samples positive	Percentage
Staphylococcus aureus	50	20	40%
Streptococcus	50	9	18%
Enterococcus	50	21	42%
Total		50	100%

Table 4. Results of multi-drug resistance tested against bacterial isolates.

Antibiotic Discs	Disk Abbreviation	*Streptococcus*	*S. aureus*	*Enterococcus*
Penicillin	P	100%	85%	98%
Cefotaxime	CTX	89.7%	65%	70%
Tetracycline	TE	69.4%	50%	60%
Augmentin	AMC	62.6%	45%	55%
Ciprofloxacin	CIP	54.2%	33%	50%
Imipenem	IPM	0%	20%	6%

DISCUSSION

Urinary tract infections are one of the common and major infections in the community and hospital settings. Urinary tract infections are caused by microbial invasion and subsequent multiplication in the entire urinary tract (Bano *et al.*, 2012). The occurrence of UTI is more common among women than men, because of structural differences, hormones, and behavioral changes. In the present study, it was found that the infection rate was higher in females than males. The prevalence of UTIs was higher among females than male patients (Ashaf, 2014; Sewify *et al.*, 2016). The prevalence of bacterial isolates was *Enterococcus faecalis* (42%), followed by *Staphylococcus aureus* (40%), and *Streptococcus pneumoniae* (18%). Various studies have reported the occurrence of bacteria in clinical samples (Ashaf, 2014; Iqbal and Ashraf, 2018; Sewify *et al.,*, 2016).

Antibiotics have also been called miracle drugs, but underuse and overuse of antibiotics have resulted in increasingly frequent resistance. Resistance rates are quite variable, depending on context and environment. Antibiotic resistance in bacteria isolated from human infections is increasing day by day making it a major public health and nosocomial problem (O'Connell *et al.,*, 2013; Zetola *et al.,*, 2005). So it is very important to determine the antibiotic resistance patterns in bacterial isolates from the human origin for proper and accurate prescriptions, failure of which is already a big problem in developing countries like Pakistan (Ashaf, 2014; Iqbal and Ashraf, 2018; Marra *et al.*, 2011).

In the present study, all *Staphylococcus, Streptococcus,* and *Enterococcus* were resistant to Penicillin, Erythromycin, and Amoxycillin as documented in a previous study (Olowe *et al.*, 2012). Previous studies in Pakistan have also shown very high antibiotic resistance in *Staphylococcus* against Cephalosporin, Erythromycin, and Penicillin (Aziz *et al.*, 2012).

During observing the susceptibility of Gram-positive bacterial pathogens it was noted that *Staphylococcus aureus* showed the highest sensitivity (64%) to Imipenem While measuring the zone diameter it was found that Cefotaxime, Penicillin, and Tetracycline were lying in the resistivity zone for *S. aureus* with 45% rate which showed resemblance to the previously reported study (Tankhiwale *et al.*, 2004). A previous investigation documented *S. aureus* as 89 and 61% sensitive to Gentamicin and Cephalexin, respectively, and resistance to Co-trimoxazole and Penicillin (Ahmad and Kudi, 2003). In slight contrast to this study, other researchers reported *S. aureus* as 100% sensitive to Gentamicin, Cephalosporin, and resistant to Augmentin, Nitrofurantoin (Shittu and Mandere, 1999). These differences in sensitivity pattern of *S. aureus* could be attributed to environmental factors such as the misuse and abuse of antibiotics among the general population, which has favored the emergence of resistance strains just as it could be the case in other organisms in any particular region or community (Utsui and Yokota, 1985).

Bacteria play a major role in causing health-care-related infections. The existence and spread of antimicrobial resistance in gram-positive bacteria have been well reported as a crucial problem worldwide (Fair and Tor, 2014). The continuous emergence of resistance against extended-spectrum Cephalosporin in gram-positive bacteria has always been an important issue, formerly in several resistant bacterial species and now growing quite rapidly (Soderblom *et al.*, 2010). Many micro-organisms have been able to survive for thousands of years as they proved to be capable of adapting to antimicrobial agents. They do this either through DNA transfer or spontaneous mutation. This process allows the bacteria to combat the physical attack posed by certain antibiotics, causing them to be ineffective (Francis *et al.*, 2005).

CONCLUSION

Our study documented the existence of multidrug-resistant bacteria in clinical isolates causing hazards to public health. Females are more infected with pathogenic bacteria than males. This is because of the anxiety disorder. Nowadays *Staphylococcus aureus* is more resistant bacteria to which antibiotics are more frequently prescribed and it is indicated that utilization of a particular antibiotic leads to the development of resistance by that pathogenic bacteria. Furthermore, as our evidence-based knowledge is limited, better-planned studies are immediately needed. The practice of antibiotics in our region of the world needs to be under health and opinion control with laboratory investigations to explore the intrinsic and extrinsic parameters that led to wrapping up high rates of resistance take place in the confined pathogens that might be spread to other geographical areas.

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