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Evaluation of antimicrobial resistance among the clinically isolated *S. aureus* and *E. coli* from a hospital in Karachi, Pakistan

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Abstract

The surfacing and extend of antibiotic resistance in different organisms (parasites, fungi, bacteria, and virus) is among the mainly important health troubles of the world today. The battle to antimicrobial drugs has increased at society and mostly at hospital settings. Pan-resistant strains (organisms resistant to all antibiotics), multidrug-resistant (MDR), and extensively drug resistant (XDR) is antibiotic treatment decline and it has amplified morbidity, and mortality, along with important effects on the price of remedial treatment of bacterial infectious diseases. In this study antibiotic resistance of different groups of commonly used antibiotics has been studied for clinically isolated of *S.aureus* and *E.coli* grown by Blood, Urine, and Pus samples. All samples have been collected at Memon Medical Institute (MMI) Hospital, Karachi. Sixty-four clinical specimens were tested for their antibiogram pattern with broadly used antibiotics for the treatment of bacterial infections. Results indicated a range of 0 to 100% of antibiotic resistance showing a failure in antibiotic activity against *E. coli* and *S.aureus*. Piperacillin + Tazobactam (Ta), Cefoperazone + Sulbactam (Sul), Ciprofloxacin/Ofloxacin, Co-trimoxazole, and Aztreonam (Azactum) were 100% resistance by *E.coli* isolated from pus samples whereas, *S.aureus* showed resistance against Erythromycin 60% from pus, Ciprofloxacin/Ofloxacin 57.14% from pus and urine, and Meropenem 57.14% from pus.

Keywords: Antimicrobial resistance, clinical isolates, Karachi, *E.coli* and *S.aureus*

Introduction

The capability of organisms to fight and to be resistant against antimicrobial agents is antimicrobial resistance (AMR). A range of antimicrobial elements is available, for example disinfectants, antibiotics and food preservatives which are used against microorganisms to inhibit growth or to destroy them. Available natural, synthetic and semi-synthetic agents perform distinct actions, like major alteration in growth and physiological level such as changes on cell wall production in case of β -lactams and glycopeptides, protein synthesis inhibitors like Macrolides and tetracyclines, metabolic pathway inhibitors like sulfonamide and interfering with translation and DNA replication by Fluoroquinolones (J.-Y. Kim et al, 2008; Kang CI & Song JH, 2013). Antimicrobial resistance has become severe issue globally, mostly in less developed countries. It has been estimated that 70% of antibiotic failure is going up in the Asia, makes it universal danger (Kang CI & Song JH, 2013). Pakistan is also among such rising South-Asian countries which are high in antibiotic resistance, making it the major risk in the region as well (Abrar S. et al, 2018) MDR and XDR bacteria, both were recognized in Pakistan in past year. For last decade from Pakistan, quinolones resistance has

become greater than before for Enterobacteriaceae (Yasmin F et al, 2013). In year 2016, XDR Salmonella outbreak is also an example that showed resistance to fluoroquinolones as up to 100% (Qamar FN et al, 2018). Raise in antimicrobial resistance has now become a challenge among the other health issues worldwide at present. Pan-resistant strains (resistant to all the current groups of antibiotics) and Multidrug-resistant (MDR) are product of antibiotic malfunction (Guillemot et al, 2002; Rice, L.B. 2010). Frequent exposure to antibiotics is considered as the most important factor that is influencing the emergence and extends of antibiotic resistance (Baquero F & Canton R, 2009). Naturally, bacteria are genetically able to transmit and obtain resistance to drugs, which are utilized as therapeutic agents (Cohen, M.L, 1992). Resistance to antibiotics can be considered mainly the outcome of a range of factors, as variation of the target of the drug, impermeability of the microbes to it or genetic changes including mutational changes transfer of resistance genes through plasmid and mutations in the targeted genes (Qi, L et al, 2016). The speedy occurrence of resistance is observed wide-reaching and it has endangered the effectiveness of antibiotics, which saved a great number of lives for a long time (Golkar

Z *et al.*, 2014; Congressional Research Service Report, 2005). Many decades after the treatment of earliest patient with antibiotics against bacterial infection, bacteria have once more become a threat in current era (Spellberg B & Gilbert DN, 2014). Antibiotic resistance disaster have been endorsed due to the excess use and abuse of the drugs, also the lacking of new medication development by medicinal industries (Gould IM & Bal AM, 2014; CDC, 2013; Viswanathan VK, 2013). According to world health organization (WHO) multidrug-resistant (MDR) *M. tuberculosis* infection is a serious and critical warning worldwide. The WHO reported that in 2012, 170,000 people died from drug resistant tuberculosis (TB) infections (Sengupta S *et al.*, 2014; Gross M, 2013). In past few decades, widespread overuse of antibiotics has risen up resistant strains, resulting in increased number of fatal infections and exerted an economic trouble on society. An investigation of antibiotic resistance in Pakistan presented by Bilal, H *et al.* 2021, has highlighted the AMR picture in Pakistan for last 10 years (Bilal H *et al.*, 2021). Outcomes of study have demonstrated that most of the pathogens illustrate elevated fight against usually used antibiotics.

Methodology

Sample Collection

Human blood, pus and urine samples were collected from Memon Medical Institute Hospital, Karachi. Samples were cultured on culture media for the possible bacterial growth. Blood, Chocolate and MacConkey's (Oxoid) agar was used for the characterization of organisms as gram positive and gram negative.

Identification and Differentiation

Identification of bacterial cultures was made with the help of their colonial, biochemical and morphological characteristics. Growth media like Blood agar for gram positive and MacConkey's agar was used for the gram-negative bacteria. Evaluation of biochemical characteristics was done with biochemical tests (IMViC, TSI and Sugars). Determination of morphological characteristics of isolated bacterial cultures was done by Gram staining (Anjum, F *et al.* 2019).

Analysis of antimicrobial resistance

Clinical and Laboratory Standards Institute (CLSI) guidelines were used for the resistance profiles determination using the standard Kirby-Bauer disk diffusion method (CLSI,2014). The antimicrobial agents tested were: amikacin (30 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg),

cefoperazone + sulbactam (Sul) (30 µg), cotrimoxazole(10µg), aztreonam (azactum) (30µg), gentamicin (10µg), cefradin (30µg), cefaclor (30µg), erythromycin (15µg), cefuroxime (30µg), fucidic acid (10µg), clindamycin (10µg), vancomycin (30µg) and linezolid (30µg).

Antibiotic Susceptibility

Organisms were categorized as sensitive or resistance on the basis of measurement of the zone of inhibition. Zone of inhibition was measured according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2014). Three categories were set as resistance, sensitive or intermediate by zone formation.

Results

Total 64 clinical samples were tested for their antibiogram pattern. Total of 34 samples were gram positive *S. aureus* and 30 were gram negative *E. coli*. Human blood, pus and urine sample were taken for the isolation of bacteria from which 05 from pus, 05 from blood, and 20 from urine *E. coli* isolates were collected on the other side 07 from pus, 07 from urine and 20 from blood *S.aureus* isolates were collected (table 1). Higher number of *E. coli* isolates was collected by urine sample considering as Uropathogenic *E. coli* (UPEC), whereas high count of *S. aureus* isolates were collected by blood sample (figure 1 and 2). Antibiogram analysis highlighted that a range of 0 to 100% of antibiotic resistance showing a failure in antibiotic activity against *E. coli* and *S.aureus*. Increased resistance was observed from Piperacillin + Tazobactam (Ta), Cefoperazone + Sulbactam (Sul), Ciprofloxacin/Ofloxacin, Cotrimoxazole, and Aztreonam (Azactum) as 100% in case of *E.coli* isolated from pus samples whereas, *S. aureus* showed resistance against Erythromycin 60% from pus sample, Ciprofloxacin/Ofloxacin 57.14% from pus and urine sample and Meropenem 57.14% from pus sample (table 2 and 3). Lowest/ no resistance was seen against Amikacin from blood and pus, Piperacillin + Tazobactam (Ta), Imipenem/Meropenem, Cefoperazone + Sulbactam (Sul) from blood in case of *E. coli* (figure 3). Whereas, Amikacin, Gentamicin, Cephadrine, Cefaclor, Cefuroxime, Fucidic Acid, Vancomycin, Linezolid from urine isolates and Linezolid, Chloramphenicol from pus isolates of *S. aureus* were 100% sensitive (figure 4). This study results indicated an overall resistance against two of the frequently used antibiotics for the cure of infections from the two most important and prevalent pathogenic isolates

Table 1, Sample collection

S.no	Organism	Sample			Total no of isolates
		Pus	Blood	Urine	
01	<i>E. coli</i>	05	05	20	30
02	<i>S. aureus</i>	07	20	07	34
Total Isolates		12	25	27	64

Table 2, Percentage of antibiotic resistance of *E. coli*

Antibiotic	Organism	Sample	% of Resistance	Sample	% of Resistance	Sample	% of Resistance
Amikacin	<i>E. coli</i>	Urine	10	Blood	00	Pus	00
Gentamicin			40		40		40
Piperacillin + Tazobactam (Ta)			15		00		100
Imipenem/Meropenem			15		00		40
Cefoperazone + Sulbactam (Sul)			15		00		100
Ciprofloxacin/Ofloxacin			50		40		100
Co-trimoxazole			60		60		100
Aztreonam (Azactum)			55		40		100

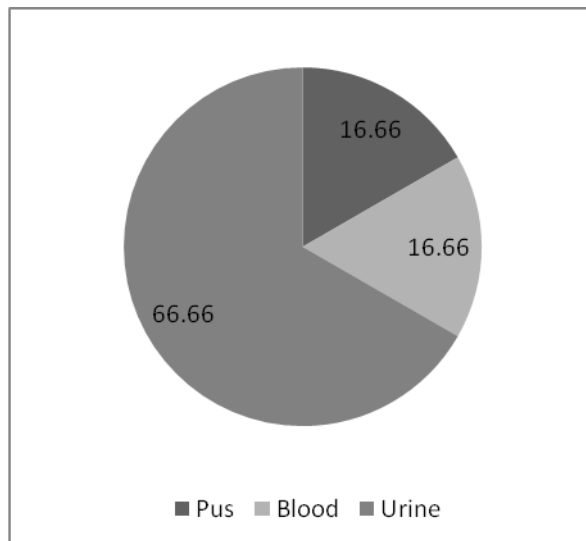


Figure 1, percentage for *E. coli* isolates from different samples

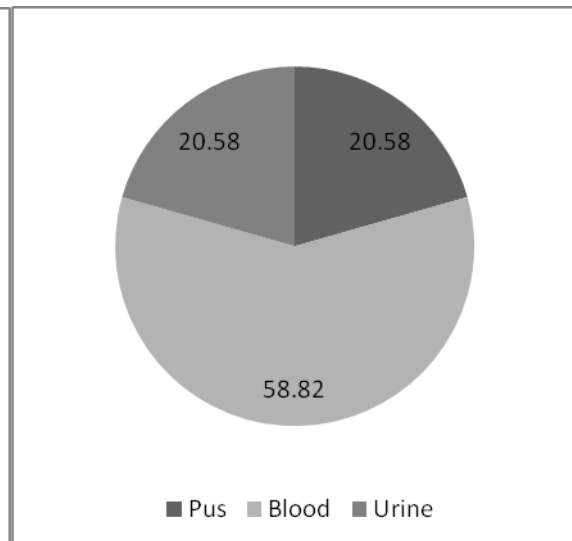


Figure 2, percentage for *S. aureus* isolates from different samples

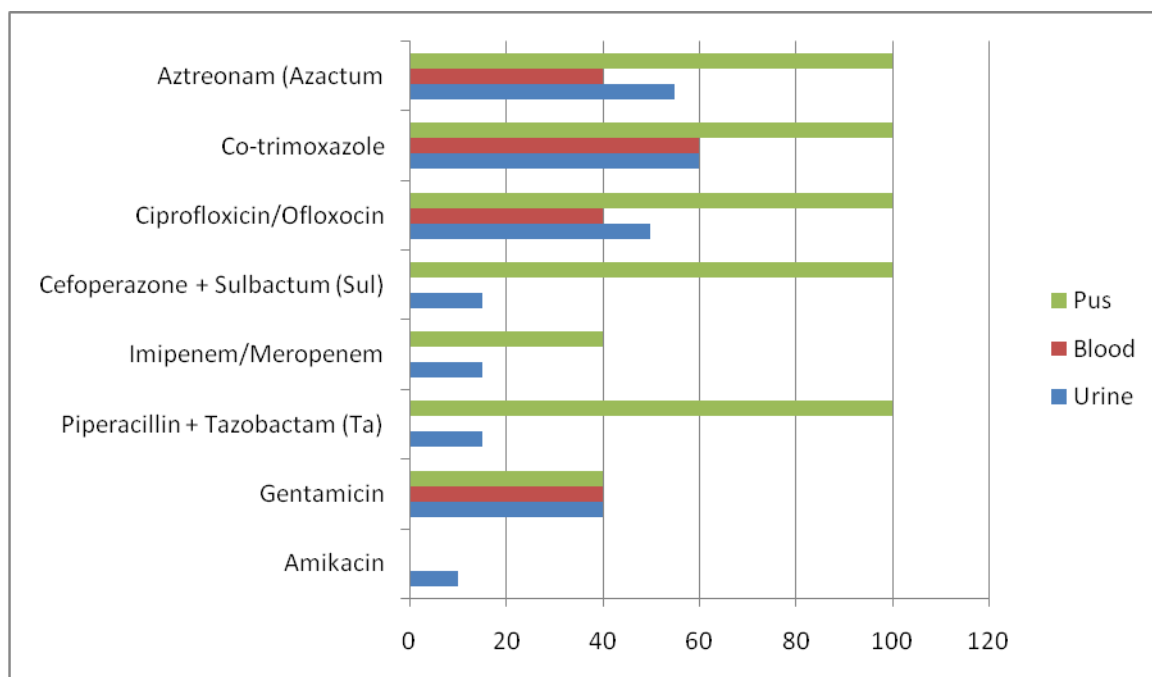


Figure 3, Percentage of antibiotic resistance of *E. coli*

Table 3, Percentage of antibiotic resistance of *S. aureus*

Antibiotic	Organism	Sample	% Percentage of Resistance	Sample	% Percentage of Resistance	Sample	% Percentage of Resistance
Amikacin	<i>S. aureus</i>	Blood	5	Urine	00	Pus	14.28
Gentamicin			15		00		14.28
Cephadrine			25		00		28.57
Cefaclor			25		00		28.57
Cefuroxime			30		00		28.57
Ciprofloxacin/Ofloxacin			30		57.14		57.14
Erythromycin			60		14.28		28.57
Chloramphenicol			15		14.28		00
Co-trimoxazole			40		28.57		14.28
Fucidic Acid			50		00		14.28
Vancomycin			25		00		14.28
Clindamycin			15		14.28		14.28
Meropenem			50		14.28		57.14
Linezolid			5		00		00

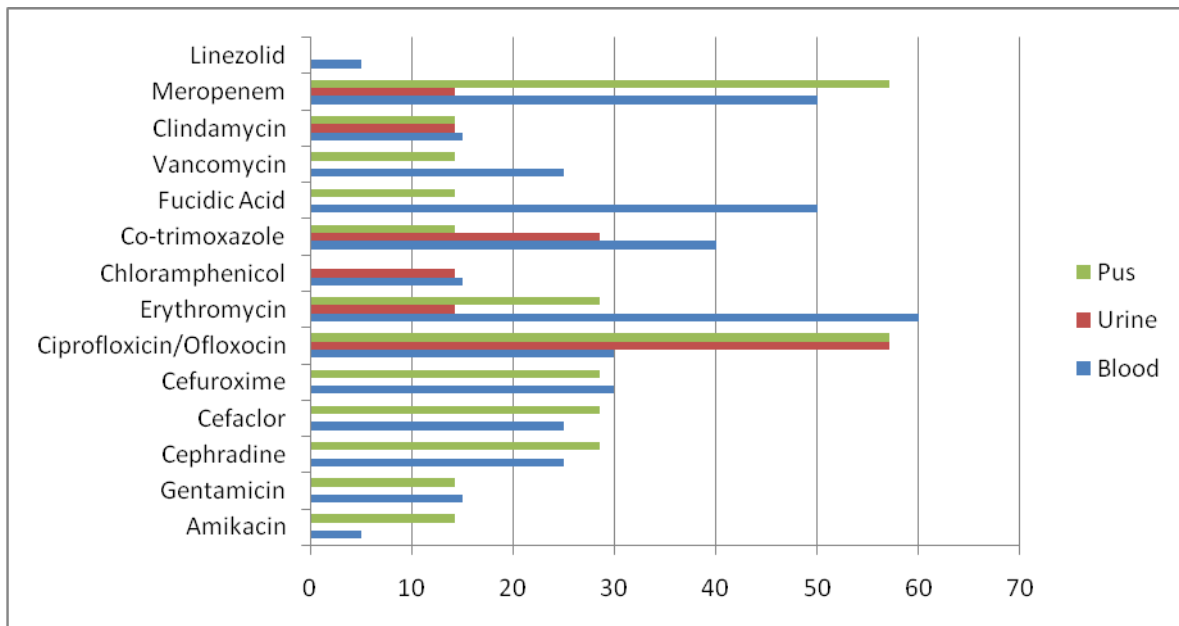


Figure 4, Percentage of antibiotic resistance of *S. aureus*

Discussion

This study was conducted to evaluate the increasing rate of antimicrobial resistance with the different groups of commonly used antibiotics. In this study, Urinary tract infections (UTI) are the highest reported finding. *E. coli* has been observed in more number of studies in bacterial pathogen viewing high tolerance to the front-line antibiotics (Bilal, H et al, 2021). It is the most common Gram-negative pathogen amongst resistant bacteria and causes a various range of diseases affecting individuals of all ages (Wu, D et al 2021). Multidrug-resistant, extensively drug-resistant and pan-drug-resistant strains of *E. coli* is now reported globally, and is among the global health issues (Shlaes DM et al, 2013; Wu, D et al 2021). *E. coli* strains can become resistant to beta lactam antibiotics by producing extended spectrum beta lactamase (ESBL), which is a plasmid-mediated β -lactamase that is capable of hydrolyzing and declining the activity β -lactam drugs like cephalosporins and monobactams (Chmlclarczyk A, 2014).

Staphylococcus aureus with other bacteria is also able to expand resistance against any antimicrobial drug to which it is treated. First discovered by the gain of β -lactamase on 'penicillinase plasmids, it was identified for several antibiotics, pre-existing greatly efficient resistance mechanism in antibiotic producers and their competitor, too easily acquired by pathogenic staphylococci by genes transfer of movable genetic elements (Nesme J & Simonet P, 2015; Kaplan SL et al 2005). A basic natural characteristic of *S. aureus* is its efficiency to colonize normally in people, asymptotically. About 30% of people are non-infectious nasal carrier of these bacteria (Kluytmans J, 1997; Gorwitz RJ et al 2008).

S. aureus is among the normal flora and its carriers are at higher hazard of infection and can be a great cause of increased pathogenic strain in people. The key form of transfer of *S. aureus* is generally through skin-to-skin touching or with direct contact of an infected individual, though contact with contaminated substance and surface is also one of the conditions (Miller LG & Diep BA, 2018; Muto CA et al, 2003)

Different host conditions like, loss of the skin obstacle, underlying health problems such as diabetes and acquired immunodeficiency syndrome (AIDS), or functional deficiency of neutrophils influences the infections. Infections by MDR strains of *S. aureus* and *E. coli* are among the other epidemics worldwide (Grundman et al 2006). The overall load of staphylococcal and UPEC disease, mainly caused by multidrug resistant (MDR) strains is increasing in both healthcare settings and in society (Chambers et al 2009). Our findings also exposed that emergence of antibiotics have been a challenge to its use for the treatment of infections as Antibiogram analysis shows 0 to 100% of antibiotic resistance expressing a failure in antibiotic activity against *E. coli* and *S. aureus*. Increased resistance from Piperacillin + Tazobactam (Ta), Cefoperazone + Sulbactam (Sul), Ciprofloxacin/Ofloxacin, Co-trimoxazole, and Aztreonam (Azactum) as 100% in case of *E. coli* isolated from pus samples is an alarming sign to the health care providers, *S. aureus* resistant against Erythromycin 60% from pus sample, Ciprofloxacin/Ofloxacin 57.14% from pus and urine sample, and Meropenem 57.14% from pus sample (table 2 and 3) refers to the decline of antimicrobial treatments.

This study is focused on antibiotic resistance, particularly in a hospital in Karachi, Pakistan. Pakistan has an important ecological position as an adjacent neighbor of the Middle East, sharing border with China, Afghanistan, Iran, India and from Uzbekistan (central Asian state) (Pakistan. Department of State publication Background notes series 1987). Drug-resistant *E. coli* and *S. aureus* have turned into a vital and composite problem in clinical treatment. This study reports a significant incidence of antimicrobial resistance in Karachi's hospital and further studies can help in finding the overall load of antimicrobial resistance to overcome this health hazard in the region. Karachi is center of trade and business in the province of Sindh and increased AMR can therefore spread at a high level if proper measures are not taken. The molecular studies for antibiotic-resistant is essential to get detailed information about the resistance mechanism (intrinsic or acquiring), which may help to design novel or substituted treatments (Munita JM & Arias CA, 1987).

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