Research Article

Antibiotic susceptibility pattern of bacterial strains isolated from different clinical samples in Multan, Pakistan

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Abstract

Rapid evolution of resistance in bacteria towards antibiotics has dramatically become a global health crisis. The aim of present study was to check the prevalence and antimicrobial sensitivity of bacteria present in clinical samples. Two hundred and seventy different clinical samples were examined in laboratory of the Chaudhary Pervaiz Elahi Institute of Cardiology, Multan Pakistan. Clinical isolates were isolated on MacConkey agar, blood agar and Cystine Lactose Electrolyte Deficient Agar (CLED Agar). The isolated strains were tested for antimicrobial susceptibility against the panel of 27 antibiotics. Out of 270 sample, 91(33.7%) showed microbial load. *Staphylococcus aureus, Pseudomonas* spp., *Escherichia coli*, *Klebsiella* spp. and *Candida* spp. were commonly isolated. *Staphylococcus aureus* showed the highest prevalence as monoresistant and polyresistant strain. The malpractice of self-medication with unrestricted supply of medicines have played an important role in antimicrobial resistance.

Keywords: Antimicrobial resistance; *Escherichia coli;* ESBL; Zone of inhibition

Introduction

World Health Organization (WHO) defines antimicrobial resistance as "resistance of a microorganism to an antimicrobial medicine to which it was previously sensitive." When microorganism develops resistance to antimicrobials, the standard treatments become ineffective thereby limiting the medical treatment of these infectious diseases. The infection persists and may spread to others [1, 2]. Failure in identification of bacterial pathogen dramatically changes the health scenario of patients and is responsible for yearly increase in deaths [3, 4]. Bacterial resistance not only has become global health epidemic but also has brutally increased the cost of treatment [4]. Poor hygiene practices and ineffective prevention measures along with misuse of antibiotics have contributed in development of resistant bacterial pathogens. Besides, insufficient number of new drugs has also worsened the situation [1].

The situation of resistant pathogens in Pakistan is alarming. Failure to provision of basic healthcare facilities and lack of awareness about hygienic life style has compromised many lives. In addition, it has added deficit in understanding and compliance of biosafety issues of community and healthcare institutions in Pakistan. The malpractice of using medicine without

consulting medical doctor has paved the path towards resistance of bacterial pathogens [5]. According to recent report of National Action Plan for Antimicrobial Resistance, excessively registered products, misleading advertisements, polypharmacy, irrational prescriptions, unrestricted availability of drugs, lack of surveillance systems and experts and widespread use of antibiotics in poultry, animals and agriculture were identified as major challenges and issues [6, 7]. As a foremost action there should be a ban on the purchase of non-prescribed drugs [8, 9]. There is a lack of active national surveillance program to establish policy legislation. Therefore, there is dire need of international and national collaborative work with government sector to address this growing uncontrolled microbial resistance in Pakistan [7]. The bacteriological profile and antibiogram pattern of patients usually vary due to different environmental factors. Therefore, this study aims to study bacteriological profile of commonly bacterial isolates from different clinical samples i.e. urine, blood, wounds, and sputum, pus, to study their antibiogram pattern and to determine prevalence of multidrug resistant (MDR) and extended spectrum beta lactamase (ESBL).

Materials and methods
Sampling and isolation
This retrospective study was conducted in Chaudhary Pervaiz Elahi Institute of Cardiology, Multan Pakistan. Laboratory records from March 2017 to March 2018 were obtained which include both demographic as well as clinical record. Two hundred and seventy samples from eight different clinical specimens were collected. These include urine, blood, sputum, pus, central venous pressure tip (CVP Tip), arterial line tip, tracheal tube and Foleys tip. For the isolation of bacteria, specimens were cultured on MacConkey agar, blood agar and cystine lactose electrolyte deficient agar.

After overnight incubation at 37ºC, bacterial growth was observed. Bacteria were characterized by colony morphology and further confirmed by gram staining and biochemical tests i.e. catalase test, oxidase test, coagulase test and germ tube test.

Antimicrobial assay
Muller Hinton (MH) agar was used for antimicrobial susceptibility testing using Kirby-Bauer Disc diffusion method [10]. This test was performed against the panel of 27 antibiotics that include Amikacin (30µg), Ampicillin (10µg), Augementin (30µg), Cefepime (30µg), Ceftazidim (30µg), Ceftriaxone (30µg), Cefuperazone (105µg), Cefuroxime (30µg), Cephradine (30µg), Ciprofloxacin (5µg), Clarithromycin (15µg), Clindamycin (2µg), Ecasil (30µg), Erythromycin (15µg), Fusidic acid (10µg), Gentamicin (10µg), Imipenem (10µg), Linezolid (30µg), Meropenem (10µg), Moxifloxacin (5µg), Nitrofurantoin (300µg), Oxacillin (1µg), Romicef (30µg), Sulzone (105µg), Tazobactum (110µg), Teicoplanin (30µg), Vancomycin (30µg).Briefly, test colony was spread evenly on MH agar and left for 5 minutes at room temperature so that inoculum get absorbed. Afterwards, commercially available antibiotic discs were impregnated aseptically and incubated at 37ºC for 24h. Zone of inhibition (mm) was measured after 24h. Depending upon diameter of zone of inhibition against each antibiotic disc, strains were classified as resistant, intermediate and sensitive. In absence of zone of inhibition, strain is considered as resistant which means that antibiotic is ineffective against test strain. In presence of zone of inhibition, the strain is considered as sensitive and antibiotic is effective against test strain.

Statistical analysis
The statistical analysis was performed using the SPSS Statistics 22.0. The distribution frequency between gender was compared. The data was also analysed seasonally i.e.

summer, winter, autumn and spring. The Chi-square test was used to compare the prevalence, sensitivity and resistance rates between different types of specimens.

Results

Clinical characteristics

Two hundred and seventy samples were examined in given time. The male to female distribution ratio was 2:1 i.e. 87(32.2%) samples were of females and 183(67.7%) were of males (Table 1). Out of 270 sample examined, 91(33.7%) showed microbial growth; 86 (94.5%) samples showed bacterial growth while 5 (5.5%) samples showed fungal growth. Among different types of clinical specimens, blood samples were the highest 116 (43%) followed by 76 (28.1%) pus, 30 (11.1%) urine, 17 (6.3%) CVP tip, 16 (5.9%) Foleys tip, 9 (3.3%) arterial line tip, 3 (1.1%) sputum and 3 (1.1%) tracheal tube samples. It was noticed that proportion of Gram-positive bacteria was higher than Gram negative i.e. 60(70%) and 26(30%) respectively. In different microbial types isolated, most prevalent bacterial type was *S. aureus* (65.93%). Other microbes isolated were *Pseudomonas* spp. (19.78%), *Candida* spp. (5.5%), *Escherichia coli (E. coli)* (5.5%) and *Klebsiella* spp. (3.3%).

Multidrug resistant (MDR) and extensive drug resistant (XDR)

Bacterial isolates that showed resistance against more than one drug were categorized as multidrug resistant (MDR) and isolates that were resistant against more than two classes of drug were categorized as extensive drug resistant (XDR). The most commonly reported MDR and XDR was *S. aureus* MDR 25 (41.67%) and XDR 35 (58.33%). In *Pseudomonas* spp. MDR and XDR were 3 (16.67%) and 15 (83.33%) respectively. However, the frequency and distribution of *E. coli* as MDR and XDR was same 1 (20%) (Table 2). Distribution of MDR and XDR isolates from males and females was merely same. The frequency of MDR in males and

females was 18 (32.73%) and 11 (35.49%) and XDR was 34 (61.82%) and 17 (54.84) (Table 3). However, the distribution of multidrug resistant (MDR) in different specimens varied. Out of 42 bacterial isolates from pus sample, 18 (42.85%) were MDR and 23 (54.76%) were XDR. From blood samples 7 (43.75%) were MDR and 9 (56.25%) were XDR. 1 (14.29%) MDR were present in Foleys tip, CVP tip and arterial line tip. 5 (71.42%), 3 (100%), 2 (28.57%), 6 (85.71%) and 3 (100%) XDR were isolated from CVP tip, Tracheal tube, Urine, Foleys tip and Sputum respectively (Table 4).

Extended spectrum beta lactamases (ESBL)

Some bacteria are resistant against beta lactamase antibiotics such as penicillin, carbapenem and cephalosporin. They were termed as extended spectrum beta lactamases (ESBL). The two main bacteria that produce ESBLs are *E. coli* and *Klebsiella* spp. All the three strains of *Klebsiella* spp. were ESBLs. While the frequency and percentage of *E. coli* as ESBL were 3 (60%) (Table 2). ESBLs was equally distributed between males and females i.e. 3 (5.45%) and 3 (9.67%) respectively (Table 3). Like MDR and XDR, the distributions of ESBL bacteria among different specimens were different i.e. 4 (57.14%), 1 (14.29%) and 1 (2.39%) respectively (Table 4).

Discussion

Antibiotics were used initially for medical applications but have expanded their use in agriculture in the 1950s [11]. It is difficult to imagine optimal health without an umbrella of antibiotics to use when needed [12]. Up to 95% of adults in India and Pakistan carry bacteria that are resistant to β-lactam antibiotics in comparison to 10% of adults in the Queens area of New York [13]. Like previously reported in this study also MDR were present with high frequency (33.7%) in clinical specimens including urine, blood, sputum, pus, central venous pressure tip

(CVP Tip), arterial line tip, tracheal tube and Foleys tip from a local hospital based diagnostic laboratory. Contradictory to Gandra *et al.* [14] who found 14% culture positive for microbial growth. This low frequency could be due to monospecimen type i.e. all samples were blood samples compared to our diverse sample types. Similar to our findings Saeed *et al.* [15] also reported common isolates *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* aeruginosa, *Klebsiella pneumoniae* and *Acinetobacter baumanii* recovered from clinical specimens [15].

Table 1. Sample characteristics

Clinical isolates	Frequency	Percentage (%)
Sex		
Male	183	67.8
Female	87	32.2
Specimen type		
Blood	116	43.0
Pus	76	28.1
Urine	30	11.1
CVP tip	17	6.3
Foleys tip	16	5.9
Arterial line tip	9	3.3
Sputum	3	1.1
Tracheal tube	3	1.1
Microbe isolated		
Bacteria	86	94.5
Fungi	5	5.5
Culture characteristics (only for bacterial isolates)		
Gram positive	60	70
Gram negative	26	30
Total	86	100
Isolated Microbe type		
S.aureus	60	65.93
Pseudomonas spp.	18	19.78
Candida spp.	5	5.5
E.coli	5	5.5
Klebsiella spp.	3	3.3

Table 2. Percentages of MDR, XDR and ESBL in different bacterial isolates

Bacterial isolates	MDR (%)	XDR (%)	ESBL (%)
n=86	n=29	n=51	n=6
S. aureus (n=60)	25 (41.67)	35 (58.33)	0
E.coli (n=5)	1 (20)	1 (20)	3 (60)
Klebsiella spp. (n=3)	0	0	3 (100)
Pseudomonas spp. (n=18)	3 (16.67)	15 (83.33)	0

Table 3. Distribution of MDR, XDR and ESBL in males and females

Gender	MDR (%)	XDR (%)	ESBL (%)
Male (n=55)	18 (32.73)	34 (61.82)	3 (5.45)
Female (n=31)	11 (35.49)	17 (54.84)	3 (9.67)

Table 4. Distribution of MDR, XDR and ESBL in different specimen types

Specimen type	MDR (%)	XDR (%)	ESBL (%)
A line tip (n=1)	1 (100)	-	-
Blood (n=16)	7 (43.75)	9 (56.25)	-
Pus (n=42)	18 (42.85)	23 (54.76)	1 (2.39)
CVP tip (n=7)	1 (14.29)	5 (71.42)	1 (14.29)
Tracheal tube (n=3)	-	3 (100)	-
Urine (n=7)	1 (14.29)	2 (28.57)	4 (57.14)
Foleys tip (n=7)	1 (14.29)	6 (85.71)	-
Sputum (n=3)	-	3 (100)	-

In our study the frequencies of MDR, XDR and ESBL were 33.73%, 59.3% and 6.97%. In the same way Sabir *et al.* [2] reported 87.17% of the positively cultured specimens in which MDR strains were prevalent with a frequency of 20.59%. *Staphylococcus aureus* was the most abundant (65.93%) higher than reported previously i.e. 30% to 15.49% [2, 16, 17]. However, *Pseudomonas* spp. in our study was 19.78% while in study of Adedeji *et al.* [17], the prevalence of *Pseudomonas* spp. was predominately about 50%. Ogisi and Osamar [18] reported a prevalence of 31% for *Pseudomonas* spp. A total of 3 (10%) MDR *Pseudomonas* spp. were reported

similar to previously reported. Likewise, Narteen *et al.* [19] also reported multi-drug resistant *Pseudomonas aeruginosa* in clsainical samples.

The prevalence of *E. coli* in our study was low (5.5%) compared to another study which reported a frequency of 26.77% [2]. Other also reported higher frequency i.e. 47.4% by Anagaw *et al.* [20], 41.7% by Hafeez *et al.* [21]. However, the frequencies of MDR in *E. coli* were 20%. The percentage of MDR *E.coli* reported by Hospenthal *et al.* [22] was 57%. The difference in findings could be due to different environment, different specimens and varying time period of research. The

3.3% frequency of *Klebsiella* spp. was much lower than reported by Latif *et al.* [23] i.e. 24.1%. None of the *Klebsiella* spp. strains isolated were MDR which is contradicted to Sabir *et al.* [2] who reported a prevalence rate of 22.58% in clinical samples e.g. tissue, urine, bone and pus samples of clinic. In this study *Candida* spp. was also isolated of about 5.5% and in the study of Sabir *et al.* [2] 26.9% of *Candida* spp. was isolated. The prevalence of Candida spp. in blood cultures was 5.8% [14]. While in our study no candida spp. was isolated from blood samples.

Conclusion

In conclusion, this study showed that *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas* spp., *Klebsiella* spp. and *Candida* spp. were the most common isolates recovered from clinical specimens. *Staphylococcus aureus* was more prevalent as compared to other isolates. The MDR strains of *Staphylococcus aureus* was high. The higher rate of ESBL strains of *Klebsiella* spp. were reported in this study. Emergent action against uncontrolled use of antibiotics is needed.

Authors' contributions

Conceived and designed the experiments: HA & HY. Performed the experiments: HA. Analyzed the data: HA & HY. Contributed reagents/materials/analysis tools: HY. Wrote the paper: HA & HY.

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