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Research Article

Prevalence and Antibiotic Resistance Pattern of Multidrug Resistant Bacteria among Blood Isolates

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Abstract: This study is conducted to determine the prevalence and antibiotic resistance pattern of multidrug resistant bacteria among blood isolates. It was conducted from July 2011 to January 2012 at Department of Pathology, Pakistan Institute of Medical Sciences, Islamabad, Pakistan. The patients referred to the laboratory for blood cultures were selected. Blood cultures were done using standard protocol. Different microorganisms were identified using standard battery of tests. Antibiotic sensitivity was checked with Kirby Bauer disk diffusion method. Blood culture was positive in 152 (14.39%) cases, out of which 58 (14.39%) were multidrug resistant bacteria. The male to female ratio was 1.76:1. Most common age group affected was between 21-40 years of age. The most common site of origin of multidrug resistant bacteria was emergency ward (26 cases; 44.87%) followed by medical ward (19 cases; 32.75%). *Staphylococcus aureus* was the most common bacteria among gram positive isolates while *Salmonella typhimurium* followed by *Pseudomonas aeruginosa* were most common amongst gram negative isolates. In gram positive isolates, *S aureus* was resistant to amoxicillin plus clavulanic acid (100%) and co-trimoxazole (53.3%) while methicillin resistant *S aureus* was resistant to amoxicillin plus clavulanic acid (100%), co-trimoxazole (81.80%) and cefuroxime (54.50%). Gram negative organisms were resistant to amoxicillin plus clavulanic acid (100%), ciprofloxacin (100%), co-trimoxazole (100%), cefepime (100%), nalidixic acid (92%) and cefoperazone plus sulbactam (71%). Our study has shown that both gram positive and gram negative bacteria are resistant to most of the commonly prescribed antibiotics and their antibiotic resistance pattern is changing.

Keywords: Multidrug resistance, antibiotic sensitivity, blood culture.

INTRODUCTION

The number of infections caused by microorganisms is increasing significantly over the last few years. One of the reasons for this increase is development of microbial resistance to drugs used to treat these infections. About 50-60% of noscomial infections in United States are now caused by antibiotic-resistant strains of bacteria [1, 2]. Multidrug resistance (MDR) microorganisms are the ones which develop resistance to the antimicrobial drugs to which they were initially sensitive [3]. Antibiotics will be ineffective against these resistant microorganisms which lead to persistence and spread of these infections. Therefore, this increase in antibiotic resistance is associated with high levels of morbidity, mortality and increased costs of treatment and hospitalization [4-6].

MDR microorganisms acquire antibiotic resistance via different mechanisms. This could be due to prevention of the drug interaction with its target, efflux of the antibiotic and drug inactivation or modification by the microorganisms [7, 8]. As a result of chromosomal mutation or horizontal gene transfer, the composition of cell membrane is altered in such a way that there will be reduced permeability and uptake of drug into the cell or the drug target sites will be modified thus rendering the drug ineffective [2, 3, 9, 10]. Efflux of the drug from within the cell is another important mechanism contributing to MDR. This is mediated by multidrug efflux pumps which expel the drug out of the cell and let the cell continue its normal function without any harm [11-13]. Hydrolysis of amide or ester bonds of antimicrobials or their chemical transformation by group transfer lead to their inactivation or degradation which contributes significantly to MDR [3, 7, 11, 14].

Multidrug resistance has posed many different challenges. It has contributed to increased morbidity and mortality and increased costs of treatment. As the conventional antimicrobials will be ineffective in the treatment of these infections, relatively newer and much expensive drugs will have to be used which increases the cost of treatment considerably [1, 2]. MDR has made the control of these diseases very difficult due to the higher chances of spread of these resistant microorganisms which not only decreases the efficacy of treatment but also leads to increased duration of infection [15, 16]. Therefore, MDR has posed significant threats to public healthcare.

This study is conducted to determine the prevalence of MDR bacteria and their antibiotic resistance pattern among blood isolates.

MATERIALS & METHODS

This study was conducted from July 2011 to January 2012 at department of Pathology, Pakistan Institute of Medical Sciences, Islamabad, Pakistan.

The patients referred to the laboratory for blood cultures were included in this study. About 5-10 ml of blood sample from adults and 2 ml from children was collected using aseptic technique. Blood sample was inoculated into Bactec Plus Aerobic/F, Bactec Plus Anaerobic/F and Bactec bottles (from Becton, Dickinson & Company, USA). They were incubated using Bactec 5070 continuous monitoring system for seven days. The criteria used for blood culture positivity included turbidity, hemolysis and clots. Positive blood cultures were Gram stained and subcultured on appropriate media. Microorganisms were identified from colony morphology and using standard tests [17].

Kirby Bauer disk diffusion method was used for bacterial antimicrobial susceptibility testing using Mueller Hinton agar. Twenty antibiotics were used in this study for susceptibility testing; i.e Amoxicillin plus clavulanic acid (AMC, 30µg), Ceftazidime (CAZ, 30µg), Ceftriaxone (CRO, 30µg), Imipenem (IPM, 10µg), Amikacin (AK, 30µg), Aztreonam (ATM, 30µg), Levofloxacin (LEV, 5µg), Cefoperazone plus sulbactam (SCF, 95/10µg), Cefepime (FEP, 30µg), Piperacillin plus tazobactam (TZP, 100/10µg), Cefoxitin (FOX, 30µg), Ciprofloxacin (CIP, 5µg), Co-trimoxazole (SXT, 1.25/23.75µg), Vancomycin (VA, 10µg), Nalidixic acid (ND, 30µg), Cefuroxime (CXM, 10µg), Gentamicin (CN, 10µg), Erythromycin (E, 30µg), Cefotaxime, (CTX, 30µg) and Meropenem (MEM, 10µg). National committee for clinical laboratory standards (NCCLS) guidelines were used for interpretation of results [18]. Bacterial isolate was said to be multidrug resistant (MDR) when the organism was resistant to at least 3 or more antibiotics. *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853 were used as control.

RESULTS

Total 1056 blood samples were collected. Out of which, bacterial growth was present in 152 (14.39%) cases while rest of 904 (85.60%) cases were negative. Out of these 152 (14.39%) positive cases, 58 (38.1%) were MDR isolates, as shown in Fig. 1.

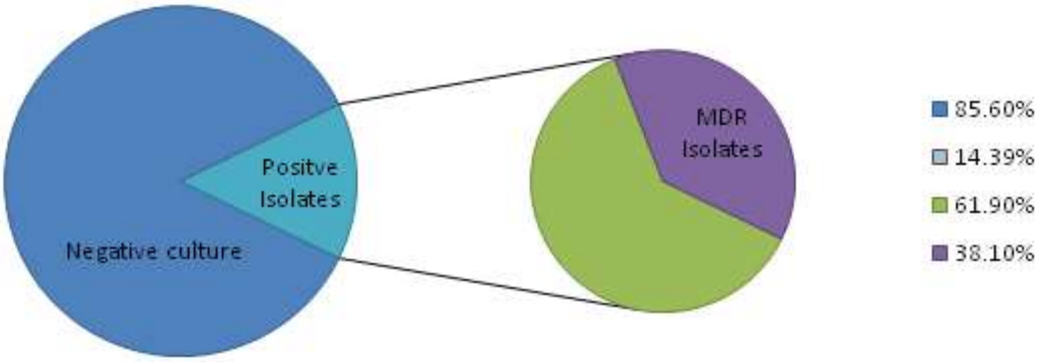

Fig. 1: Frequency of blood & MDR isolates in the study samples

There were 58 MDR isolates. Out of these, 37 (63.79%) were obtained from male patients and 21 (36.20%) from female patients as shown in Fig. 2. The male to female ratio was 1.76:1.

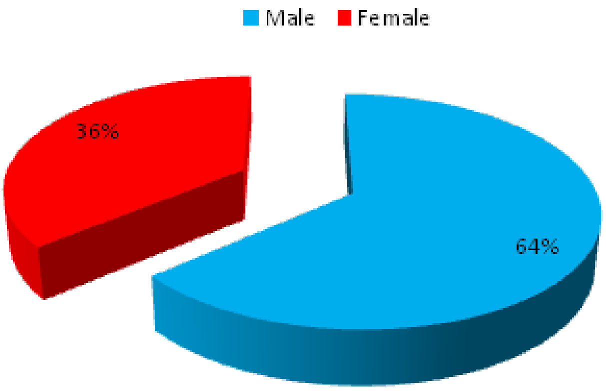

Fig. 2: Gender distribution of MDR isolates

According to the site of origin or location, the MDR isolates were most prevalent in emergency ward as there were 26 (44.87%) cases, followed by medical ward with 19 (32.75%) cases, general medical ward water filter with 7 (12.06%) cases and the medical intensive care unit (ICU) with 4 (6.89%) cases. MDR isolates were least prevalent in surgical and nephrology wards with one (1.72%) case in each of these wards as shown in Fig. 3.

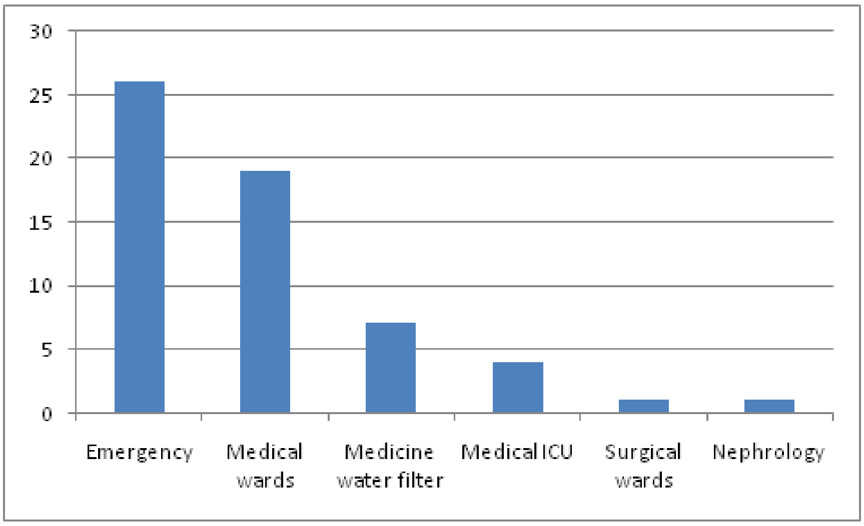

Fig. 3: Frequency of MDR isolates from different hospital sites

The distribution of MDR bacteria in different age groups was also studied. It was found out that the incidence of MDR bacteria was most common in age group between 21-40 years with 23 (38.90%) cases followed by 0-20 and 41-60 years age groups with 15 (25.40%) and 13 (22%) cases respectively. The least no of MDR positive isolates were seen in elderly patients, above the age of 61 who had 7 (12.06%) cases as shown in Fig. 4.

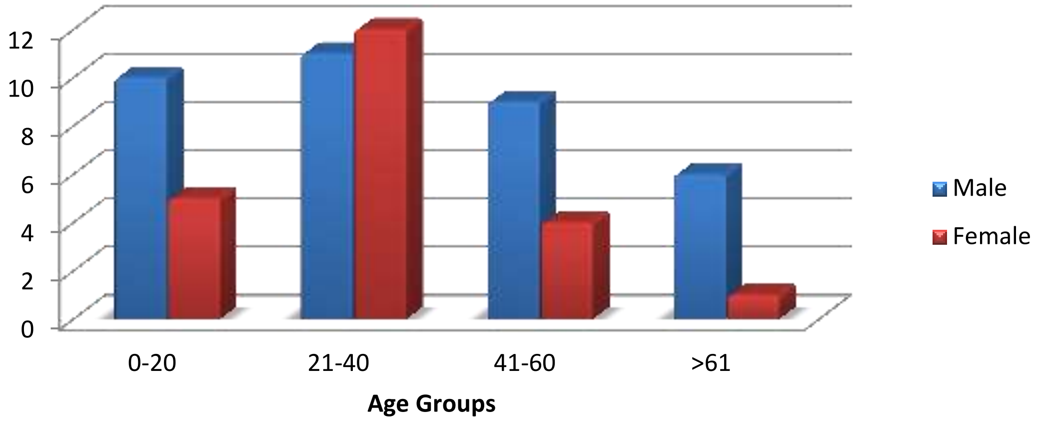

Fig. 4: Distribution of MDR isolates in different age groups

There were 152 cases of culture positive bacterial isolates. The prevalence of bacterial isolates according to gram staining was shown in Table 1. There were 104 (68.42%) cases of gram negative isolates and 48 (31.57%) cases of gram positive isolates.

Table 1: No of bacterial isolates in study sample according to Gram staining

Pathogen	**No of cases**	**Percentage**
Gram Negative Isolates	104	68.42%
Gram Positive Isolates	48	31.57%
Total	152	

S aureus was the commonest bacteria among gram positive isolates while *S typhimurium* and *P aeruginosa* were commonest among gram negative bacteria followed by *E coli* and *Klebsiella pneumoniae* as shown in Table 2.

Table 2: Distribution of culture positive bacterial isolates

	No of Isolates	**%**
Gram Positive Bacteria		
Staphylococcus aureus	30	19.7
Methicillin Resistant *S aureus* (MRSA)	11	7.23
Streptococcus viridans	7	4.6
Gram Negative Bacteria		
Salmonella typhimurium	39	37.5
Pseudomonas aeruginosa	23	22.1
Escherichia coli	18	17.3
Klebsiella pneumoniae	14	13.4
Acinetobacter	2	1.92
Enterobacter agglomerans	2	1.92
Proteus mirabilis	2	1.92
Salmonella paratyphi A	1	0.96
Enterobacter cloacae	1	0.96
Serratia marcescens	1	0.96
Enterobacter spp.	1	0.96

The susceptibility of bacteria to different antibiotics is shown in Table 3 & 4. Among gram positive organisms, *S aureus* was resistant to amoxicillin plus clavulanic acid (100%) and co-trimoxazole (53.3%) while it was sensitive to cefuroxime (80%) and azteonam (70%). Mehticillin resistant *S aureus* (MRSA) was resistant to amoxicillin plus clavulanic acid (100%), co-trimoxazole (81.80%) and cefuroxime (54.50%). Gram negative organisms were resistant to amoxicillin plus clavulanic acid (100%), ciprofloxacin (100%), co-trimoxazole (100%), cefepime (100%), nalidixic acid (92%) and cefoperazone plus sulbactam (71%) but they were sensitive to levofloxacin and imipenem except for *Acinetobacter* .

Table 3: Antibiotic susceptibility of gram positive blood isolates (% resistance)

Sl. No.	**Antibiotics/organism**	**Cefuroxime**	**Aztreonam**	**Co-trimoxazole**	**Amoxicillin plus clavulanic acid**
1	***S aureus***	20%	30%	53.30%	100%
2	**MRSA**	54.50%	45.40%	81.80%	100%

Table 4: Antibiotic resistance of gram negative blood isolates (% resistance)

Antibiotic	***E coli* (18)**	***K pneumonae* (14)**	***P aeruginosa* (23)**	***Acinetobacter* (2)**
Ciprofloxacin	100%	0	100%	0
Cefepime	100%	100%	100%	100%
Co-trimoxazole	100%	0	100%	0
Nalidixic acid	92%	0	0	0
Cefoperazone plus sulbactam	71%	71%	0	0
Amoxicillin plus clavulanic acid	44.40%	78.50%	0	50%
Ceftazidime	44.40%	78.50%	16.60%	100%
Aztreonam	44.40%	57.10%	9.09%	50%
Levofloxacin	16.60%	21.40%	13.60%	0
Imipenem	0	0	4.54%	50%

DISCUSSION

This study was conducted to determine the prevalence and antibiotic resistance patterns of multidrug resistant bacteria among blood isolates.

Our study showed that the highest number of positive isolates was present in the patients between the ages of 21-40 years. This is in contrast to Shah *et al.*, who reported that the common age group was 50-60 years [19].

In this study, the blood culture was positive in 152 (14.39%) cases whereas Arora and Devi and Ayobola *et al.* have reported culture positivity to be 20.02% and 19.3% respectively [20, 21]. This variation may be due to the fact that most of the patients use antibiotics before coming to hospital and also as a result of self-medication because these medicines are available over the counter.

The incidence of gram positive organism in our study was 31.57% (48 cases) while that of gram negative organism was 68.42% (104 cases). This is in accordance with other studies that have shown that the incidence of gram negative organisms is more than the gram positive organisms [1] [22, 23]. Among gram positive organisms, *S aureus* was isolated in 30 (19.7%) of the cases while there were 11 cases (7.84%) of MRSA. Jones has reported the incidence of *S aureus* to be 23% in USA [1]. Roy *et al*. reported the incidence of *S aureus* to be 14% while that of MRSA to be 16.5% [24]. Kumar *et al*. reported the incidence of both *S aureus* and MRSA to be 9% [25].

Among gram negative organisms, *P aeruginosa* was isolated in 23 (15.7%) of the cases, *E coli* in 18 (11.84%) and *K pneumonia* in 14 (9.21%) of the cases. Jones reported the incidence of *P aeruginosa*, *E coli* and *K pneumonia* to be 18.2%, 4.4% and 8.7%, Roy *et al.* reported the incidence of these organisms to be 2.9%, 14% and 24.5% and Mehta *et al*. reported their incidence to be 19.75%, 15.17% and 14.99% respectively [1, 23, 24]. This difference in the incidence of these organisms may be due to the fact that the choice of antibiotic therapy and epidemiology of causative organisms is different among different hospitals.

Enterobacter spp. was present in 4 (2.63%) cases and *Acinetobacter* in 2 (1.31%) cases. Arora & Devi reported the incidence of *Enterobacter* spp. to be 14.19% while that of *Acinetobacter* to be 6.99% [20]. Ayobola *et al.* have reported the incidence of *Enterobacter* spp. to be 11.1% while Chhina and Gupta have reported the incidence of *Acinetobacter* to be 4.3% [21, 26].

Our study has shown that among gram positive organisms, *S aureus* was resistant to amoxicillin plus clavulanic acid (100%) and co-trimoxazole (53.3%) while they were sensitive to cefuroxime (80%) and aztreonam (70%). MRSA were resistant to be amoxicillin plus clavulanic acid (100%), co-trimoxazole (81.80%) and cefuroxime (54.50%). Edoh and Alomatu have reported the same finding in their study in which isolates were highly resistant to penicillins and co-trimoxazole [27]. Arora and Devi also reported ampicillin resistance to be 74.61% in their study [20]. Gram negative bacilli were resistant to amoxicillin plus clavulanic acid (100%), ciprofloxacin (100%), co-trimoxazole (100%), cefepime (100%), nalidixic acid (92%) and cefoperazone plus sulbactam (71%) but they were sensitive to levofloxacin and imipenem except *Acinetobacter* . Ayobola *et al*. have also reported 100% resistance to amoxicillin plus clavulanic acid in their study which is same as we have found out in our study [21]. Our study has shown that the gram negative organisms were resistant to ciprofloxacin (100%) which is in contrast to what Aybola *et al.* and Mehdinejad *et al.* reported. They have shown in their studies that the gram negative organisms were sensitive to quinolones [21, 28]. This may be due to the changing pattern of antibiotic use as ciprofloxacin is available and used for quite sometime so the microorganisms have developed resistance to it over time. This is further confirmed by the fact that gram negative organisms showed high resistance to ciprofloxacin in our study but they were sensitive to levofloxacin which is a relatively newer drug in quinolone family.

Our study has shown that both gram positive and gram negative bacteria are resistant to most of the commonly prescribed antibiotics, their antibiotic resistance pattern is changing. In comparison to other studies, the no of multidrug resistant bacteria is increasing. Therefore, there is a need to conduct such studies regularly to know the epidemiology and antibiotic sensitivity patterns of blood-borne infections to guide clinicians in their choice of empirical antibiotic therapy. As most of these organisms are acquired from hospitals, infection control measures should be strictly enforced to prevent the spread of these infections.

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