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Prevalence of multi-drug resistant bacterial pathogens among body fluids

Shamama Mateen, Abdul Wahab and Zaid Ahmed Pirzada*

M.Sc, Department of Microbiology, University of Karachi

Assistant Professor, Department of Microbiology, University of Karachi

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ABSTRACT

Objectives: The aim of the present research study was to evaluate the prevalence and antibiotic resistance pattern of the scarcely investigated invasive pathogens causing body fluid infections. **Design:** The study was conducted in the Department of Microbiology, University of Karachi, from July-December 2013. **Methods:** In this study 21 samples of body fluids were collected from different hospitals and pathologic laboratories of Karachi, among which 11 samples were of peritoneal fluid, 8 of pleural fluid and 2 of cerebrospinal fluid. Isolates were tested against gentamicin, ciprofloxacin, cefotaxime, ceftriaxone, erythromycin, penicillin G, Amoxycillin/clavulanic acid and amikacin through Kirby-Bauer disk diffusion method for antibiotic susceptibility testing and broth macrodilution technique was performed to determine their minimum inhibitory concentration (MIC) levels. **Results:** Gram negative organisms were isolated pre-dominantly (86%) from these specimens. The most frequent gram negative organism was *Pseudomonas aeruginosa* (28%) followed by *Klebsiella pneumoniae* (24%) and *Escherichia coli* (19%). Resistance to Amoxycillin/clavulanic acid was 86%, gentamicin, cefotaxime, ceftriaxone was 57%, ciprofloxacin 43% while the least resistance was for amikacin i.e. 24%. MIC of the antibiotics among isolated pathogens indicated a high level of resistance against Cefotaxime, Ceftriaxone and Amoxicillin-clavulanic acid (>1024 µg/mL) while 1024 µg/ml against Gentamicin. Altogether, 71% pathogens isolated in this study were resistant to more than one antibiotic. **Discussion and Conclusion:** The results of this study indicate that amoxicillin/clavulanic acid and third generation cephalosporins have decreased their efficacy for the empirical treatment of spontaneous bacterial peritonitis. The reason for this increased resistance among invasive pathogens might be linked with the emergence of multi drug resistance among the normal flora of bowel.

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1. Introduction

Invasive bacterial infections like spontaneous bacterial peritonitis (SBP), pleural effusions or empyema and bacterial meningitis are the most important causes of morbidity and mortality through out the world. Peritoneal, pleural and cerebrospinal fluids are the common sterile body fluids submitted for culture in laboratories. As the body fluids are normally sterile, the organisms that grow, regardless of the quantity, are worked up with an identification and antibiotic sensitivity pattern.

Spontaneous bacterial peritonitis is one of the most frequent and serious complication in patients with liver cirrhosis and ascites, associated with high mortality and comprising 31% of all bacterial infection.[1,2] Cirrhotic patient with ascites are particularly susceptible to spontaneous bacterial peritonitis due to altered gut permeability, suppression of the reticuloendothelial system and

bacterial overgrowth.[3,4] Prevalence of SBP is 10-30% in cirrhotic patients with ascites admitted to hospitals in the West 5-8 while it is around 33% in Pakistan.[9, 10]

More than 92% of all cases of SBP are monomicrobial, with aerobic gram-negative bacilli being responsible for more than two thirds of all cases. *Escherichia coli* accounts for nearly half of these cases, followed by *Klebsiella* species and other gram negative bacteria.[11,14] The infecting organisms in peritoneal fluid are usually enteric Gram negative organisms which have translocated from the bowel.[3]

Pleural fluid is secreted by the parietal layer of the pleura and collected through the process of thoracentesis. Bacteriology of pleural effusion is diverse and comprises of a complex mixture of aerobes and obligate anaerobes. Pleural effusions and empyema also require prompt diagnosis and aggressive treatment with appropriate antibiotics. Cerebrospinal fluid is collected to diagnose meningitis, as it involves the central nervous system therefore is of critical importance. At least 125000 infants and children die from bacterial meningitis yearly while 96% of these are in developing countries. The most common pathogens recovered are *Spneumonia*, *N meningitis* and *H influenzae*.

* Corresponding Author: **Dr Zaid Ahmed Pirzada**

Assistant Professor,
Department of Microbiology,
University of Karachi

Email address: zaahmed@uok.edu.pk

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Several recent reports have demonstrated the emergence of resistance to antibiotics among bacterial pathogens isolated from body fluids.^{15, 16} Amoxicillin-clavulanic acid and third- generation cephalosporins are considered as the first choice empiric antibiotic in SBP treatment, since cefotaxime has 100% penetration into ascitic fluid with no risk of nephrotoxicity. [3, 11] But a switch to another antibiotic was found essential in more than 40% of cases. [17] *E.coli*, the most frequent pathogen of peritoneal fluid, were reported to show significant resistance against amoxicillin-clavulanic acid. [18, 19]

Consequently, it is important to isolate pathogens and identify resistance patterns accurately, for prescribing appropriate antibiotics for resistant bacteria and directing standard policies in all similar settings. The infections caused by multi-drug resistant organisms are responsible for the lengthy stay at hospital, treatment with more expensive drugs and increased risk of death. The aim of this research study was to gain knowledge about invasive bacterial pathogens causing body fluid infections and to evaluate their resistance pattern from the laboratories and the hospital of Karachi.

MATERIAL AND METHODS

Collection of clinical samples

In this study a total of 21 Pleural fluid, peritoneal fluid and CSF samples were collected from different pathological laboratories and hospitals of Karachi from July-December 2013.

Identification of isolates

Gram negative organisms were preserved in 20% glycerol while Gram positive organisms were preserved in 17% glycerol after overnight growth on appropriate media. All the pathogens were identified on the basis of colonial, microscopic and biochemical characteristics with the help of Bergey's Manual of Systematic Bacteriology Vol. 3: The Firmicutes.

Antibiotic susceptibility testing

Kirby-Bauer disk diffusion method was used for antimicrobial susceptibility testing. Antimicrobial susceptibility tests were done on Mueller-Hinton agar (Oxoid, UK). The organisms were tested against erythromycin (10 µg), penicillin G, gentamicin (10 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), ceftriaxone (30 µg), Amoxycillin/clavulanic acid (30 µg) and amikacin (30 µg). Growth inhibition zone diameters were measured in millimeters and results interpreted as recommended by the Clinical laboratory standards institute (CLSI) guidelines 2013.

Minimal inhibitory concentration (MIC) determination

Broth macrodilution technique was performed using nutrient broth to determine MIC. For the interpretation of MIC, breakpoints were referred from CLSI guidelines 2013.

RESULTS

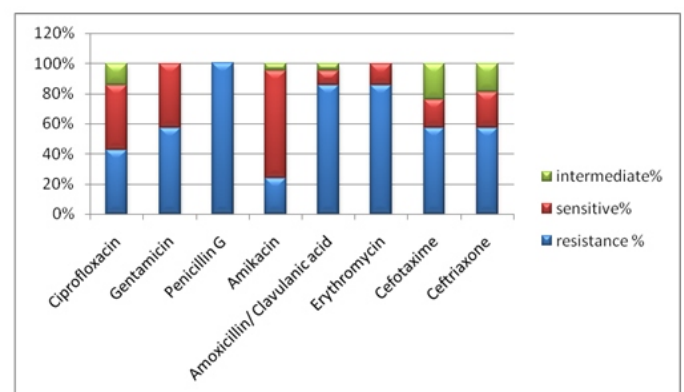
Altogether 21 samples of body fluids were collected from different hospitals and pathologic laboratories of Karachi. Among these 11 samples were of peritoneal fluid, 8 of pleural fluid and 2 of CSF. Gram negative organisms were isolated pre-dominantly 18 (86%) from these specimens. Three gram positive organisms were identified as *Staphylococcus aureus* from Pleural fluids. The most pre-dominant gram negative organism was *Pseudomonas aeruginosa* (6) isolated from all the kinds of samples collected in this study. Followed by *Klebsiella pneumonia* (5) and *Escherichia coli* (4) (Table 1).

Table 1 Distribution of pathogens among sterile body fluids

Organisms	Body Fluids				
	Asitic fluid	Pleural fluid	CSF	Total	%
<i>Escherichia coli</i>	4	-	-	4	19
<i>Klebsiella pneumoniae</i>	4	1	-	5	24
<i>Pseudomonas aeruginosa</i>	2	2	2	6	28
<i>Staphylococcus aureus</i>	-	3	-	3	14
<i>Morganella morganii</i>	1	-	-	1	5
<i>Stenotrophomonas maltophilia</i>	-	1	-	1	5
<i>Proteus mirabilis</i>	-	1	-	1	5
	11	8	2	21	100

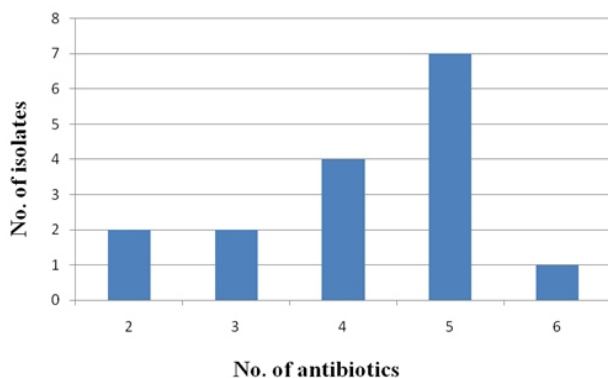
All the pathogens isolated from body fluids were tested against antibiotics belonging to the various groups to determine their antibiotic resistance pattern. Gram negative organisms are intrinsically resistant to penicillin G and erythromycin but penicillin G was not effective against gram positive organisms as well. However, erythromycin was effective for gram positive isolates. Resistance to Amoxicillin/clavulanic acid was 18 (86%), gentamicin, cefotaxime, ceftriaxone 12 (57%), ciprofloxacin 9 (43%) and amikacin 5 (24%) (Figure 1). Amikacin was observed the most effective antibiotic in this study. Except five isolates, all the organisms were sensitive to amikacin.

Figure 1 Antibiotic susceptibility pattern of organisms isolated from body fluids



The multi drug resistance analysis among isolates of body fluids revealed that one of the isolated strain of *Pseudomonas aeruginosa*, recovered from peritoneal fluid was resistant against 6 different antibiotics, while 7 other pathogens were resistant against 5 antibiotics and 4 pathogens were resistant against 4 antibiotics indicating the prevalence of multi drug resistant pathogens among invasive infections (Figure 2).

Figure 2 Multi drug resistance analysis



In order to determine the level of resistance among isolated pathogens, minimum inhibitory concentration (MIC) of the antibiotics were observed. A high level of resistance was found against Cefotaxime, Ceftriaxone and Amoxicillin-clavulanic acid (>1024 µg/mL, Table 2). Significant resistance was also observed against Gentamicin and Ciprofloxacin (1024 and 128 µg/ml respectively) by the isolated pathogens. However, Gram positive organisms did not show a high level of resistance.

Table 2 MIC of invasive pathogens against antibiotics

Antibiotics (No. of Strains)	Range (µg/ml)	MIC (µg/ml)	MIC ₅₀ (µg/ml)	MIC ₉₀ (µg/ml)	Breakpoint (S/R)	Level of Resistance (No. of times)
Cefotaxime (11)	4 - 1024	>1024	>1024	>1024	≤8 / ≥64	16
Ceftriaxone (12)	4 - 1024	>1024	>1024	>1024	≤8 / ≥64	16
Gentamicin (11)	4 - 1024	1024	512	1024	≤4 / ≥8	128
Ciprofloxacin (8)	2 - 256	128	64	128	≤1 / ≥4	32
Amoxicillin-clavulanic acid (17)	4 - 1024	>1024	1024	>1024	≤4 / ≥16	64
Amikacin (5)	32 - 1024	>1024	64	>1024	≤16 / ≥32	32
Penicillin G (3)	0.25 - 16	1	8	1	≤0.125 / ≥0.25	4

DISCUSSION

The bacterial resistance to one or more antibiotics has increased to the point that previously effective antibiotics are no longer useful against certain types of bacteria. Several recent studies have reported the emergence of resistance to antibiotics among bacterial pathogens isolated from body fluids. 15,16,18,19,20 Similarly, this study has also indicated a high level of resistance against antibiotics by the invasive pathogens of body fluids from Karachi, Pakistan.

Particularly a high level of resistance is observed against cefotaxime and ceftriaxone, which were considered as the drugs of choice for empirical treatment and prophylaxis of SBP 11,12. Some previous studies have also shown the emergence of significant resistance to third-generation cephalosporins by bacterial isolates²⁰, indicating that these drugs should not be used as the first-line antibiotics for SBP treatment anymore, since switch to another antibiotic was essential in more than 40% cases 17.

According to The International Ascites Club, amoxicillin-clavulanic acid can also be used for the empirical treatment of SBP²¹. *Escherichia coli*, that accounts for nearly half of the cases of SBP, has been reported to show significant resistance against amoxicillin-clavulanic acid^{16,18}. Similarly frequency of SBP was 56% from Pakistan (Zaman et al., 2011) among which *Escherichia coli* was the most frequently recovered organism (66%). This study has also signified a high level of resistance against amoxicillin-clavulanic acid by the isolated Gram negative pathogens including *Escherichia coli*. In the light of these observation, it has been indicated that third-generation cephalosporins and amoxicillin-clavulanic acid has decreased its efficacy for the empirical treatment and prophylaxis of SBP.

Moreover, 15 organisms isolated in this study were found resistant to more than one antibiotics and most of the pathogens were recovered from peritoneal fluid. Since the infecting organisms in peritoneal fluid are usually enteric gram negative organisms which have translocated from bowel 2, this study might be pointing towards the emergence of multi drug resistance among the normal flora of bowel due to the indiscriminate use of broad spectrum antibiotics for empirical treatment of mild to severe bacterial infections. To confirm the emergence of multi drug resistance among the normal flora of bowel, further studies are suggested as the phenomenon of drug resistance among normal microbial flora is detrimental in many ways. Specially endogenous infections could become a serious problem for public health due the emergence of drug resistance among normal microbial flora.

The alarming level of emerging resistance to antibiotics indicates an urgent need to find alternate options of therapy. Combination therapy for treatment could be an approach to prevent or delay the emergence of resistance during antimicrobial therapy. As an alternative therapeutic options, bacteriocins and herbal compounds can be explored. Furthermore probiotics can also be used for combating bacterial infections specially for the treatment of SBP, by reequilibration of intestinal flora.¹³ Another approach employs bacteriophages, for the treatment of infections by specific bacterial pathogens.

Knowledge of the incidence rate, microbial spectrum of infections and antibiotic resistance in a particular population is an essential part for the selection of the most appropriate empiric antibiotic regimen for the treatment of such infections. Regular monitoring of susceptibility to various antibiotics of pre-dominant bacterial organisms isolated from clinical sources as well as from normal microbiota should also be done by health care centres in order to prevent the emergence and spread of drug resistant organisms.

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