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Antibiogram of gram-negative bacteria isolated from in-house patient samples in a tertiary care hospital in Kolkata

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Abstract

Emerging resistance in Gram-negative bacteria and antibiotic use pattern are major concerns in clinical settings. This study aimed to detect common gram-negative bacteria and their antibiotic resistance patterns within 4 consecutive months in a tertiary care hospital in Kolkata. We determined the prevalence and antibiotic resistance patterns of bacterial isolates from blood, sputum, wound swab, urine and stool from February to May of 2015 at the adult ward in Fortis Hospital, Anandapur, and Kolkata. Identification of bacteria was done by the biochemical tests such as nitrate, indole, oxidase and urease. Antimicrobial resistance screening was performed by using 13 antibiotics, by the Kirby Bauer disk diffusion technique. *E. coli* was the most prevalent pathogen (38.35%) followed by *Klebsiella* (26.31%), *Pseudomonas* (21.05%), *Acinetobacter* (14.29%). 33.08% of the isolated gram negative organisms were found to be multidrug resistant. In our study MDR is high in *E. coli* (43.14%) followed by *Pseudomonas* (28.57%), *Acinetobacter* (26.31%), *Klebsiella* (25.71%). Antibiotic resistivity pattern of 7 different antibiotic groups were done in this study, in which most effective drug compared to other was found to be cephalosporins with an average of approx. 6.7% resistivity in all organisms. Overall, our findings indicate the prevalence of resistance to different classes of antibiotics in bacterial isolates from different infections and hence highlight the need for effective surveillance, regulator reporting, and antibiogram-guided antibiotic prescription.

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Introduction

Infections caused by antibiotic resistant bacteria are a significant cause of morbidity and mortality worldwide. According to the Centers for Disease Control and Prevention (CDC) estimation, antibiotic resistance is responsible for more than 2 million infections and 23000 deaths each year in the United States. It has been also estimated that more than 70% of the bacteria that causes hospital acquired infections are resistant to at least one antibiotic (Muto, 2005).

Infectious diseases are common cause of morbidity and considered as the second cause of mortality in chronic kidney disease patients (Samanipour *et al.,* 2016). Sepsis-related death among hemodialysis patients is 100 times more than the general population (Sarnak and Jaber, 2000). On the other hand, urinary, respiratory, and hemodialysis catheter-related bloodstream infections are common among patients with renal diseases (Samanipour *et al.,* 2016).

Like gram-positive pathogens, mainly *Staphylococcus aureus* and *Staphylococcus epidermidis,* gram-negative bacteria are also associated with an increase in rates of antibacterial resistance in catheter-related infections. Among gram negatives, extended-spectrum β-lactamase (ESBL), carbapenem-resistant, and multi-drug resistant Enterobacteriaceae specially pose a serious threat to patients in healthcare settings.

Most low and middle-income countries such as India lack national continuous surveillance systems for antibiotic resistance. Having awareness of antimicrobial resistance pattern can lead to selecting an optimized antimicrobial agent or regimen and consequently, minimizing duration of hospitalization, morbidity, mortality, and direct as well as indirect health care costs.

Gram-negative bacteria are significant causes of serious infections, and many of the most important members of this family are becoming increasingly resistant to currently available antibiotics. This is a troubling trend, and one that requires vigilance and intensified measures to control the further spread of resistance by these important gram-negative pathogens. Both improvements in antibiotic use and infection control strategies are needed before we have to deal with infections caused by multidrug-resistant gram-negative pathogens.

Materials and methods

Study area and sample collection

The study was conducted in the Bacteriology Laboratory, School of Studies in Microbiology, Jiwaji University, Gwalior, and Madhya Pradesh, India. During a 4-month period from February to May of 2015 at the adult ward in Fortis Hospital, Anandapur, Kolkata, bacteriological data of all biological samples of in-house patients in favor of gram-negative microorganisms were analyzed retrospectively. The samples studied were collected from isolates including blood, urine, sputum, wound swab and stool taken as antibacterial treatment infections brought to the laboratory of the Department of Microbiology, Jiwaji University, Gwalior, and Madhya Pradesh, India for the isolation and characterization.

Characterization of the selected isolates

The selected isolates were characterized by morphological and biochemical methods. Morphological characteristics such as colony size, shape, colour etc. were observed. Smears of suspected colonies were stained with Gram's stain and examined morphologically for staining characters. Motility was tested under light microscope of 100 magnifications by using slide with a drop of young bacteria.

Antimicrobial agents

The following antibiotic discs (per unit disc) were used for antimicrobial susceptibility of isolated gram-negative bacteria: Norfloxacin (10 mcg/disc), Ofloxacin (5 mcg/disc), Nalidixic Acid (30 mcg/disc), Ciprofloxacin (5 mcg/disc), Amikacin (30 mcg/disc), Amoxycillin (30 mcg/disc), Ampicillin (10 mcg/dis), Cloxacillin (10 mcg/disc), Tetracycline (30 mcg/disc), Cotrimoxazole (25 mcg/disc), Cephalexin ((30 mcg/disc), ceftriazone (30 mcg/disc) and Chloramphenicol (30 mcg) were used. Interpretation of the results was done using the zone of inhibition sizes provided by the antibiotic disc manufacturer (Hi-Media, Mumbai, India).Isolates simultaneously resistant to 2 or more drugs were considered as multidrug resistant (MDR).

Biochemical characterization

Identification of bacteria was done by the additional biochemical tests such as nitrate, indole, oxidase and urease. Antibiotic susceptibilities of bacterial isolates were determined according to the method recommended by the Clinical and Laboratory Standards Institute. Briefly, inocula were prepared for each bacterial isolate by adjusting the turbidity to 0.5 McFarland standard and spread on Muller-Hinton agar plates. Antibiotic discs (HiMedia, Mumbai, India) were placed on the agar plates and incubated overnight at 37°C for 24 h. The zones of inhibition were measured and the isolates were classified as sensitive, intermediate, and resistant according to CLSI tables and guidelines.

Results

Total of 408 of the clinical samples received in a period of 4 months (February-May, 2015) where 133 (32.6%) of the samples were gram negative organisms. Total of 4 different gram negative isolates were identified, distribution of the isolates in the sample is listed in Table 1.

Table 1. Number of various gram negative bacteria isolated from different specimens.

Organisms	Specimens					Total	Percentage
	Blood	Sputum	Wound Swab	Urine	Stool		
E.coli	5	16	10	17	3	51	38.35
Klebsiella	14	10	6	5	0	35	26.31
Pseudomonas	10	6	5	3	4	28	21.05
Acinetobacter	6	5	2	3	3	19	14.29
Total	35	37	23	28	10	133	100

The 4 most common isolated gram-negative bacteria from all biological samples of patients where *E. coli* was the most prevalent pathogen (38.35%) followed by *Klebsiella* (26.31%), *Pseudomonas* (21.05%), *Acinetobacter* (14.29%). Antibiotic resistivity pattern of 7 different antibiotic groups were done in study, in which most effective drug compared to other was found to be cephalosporins with an average of approx. 6.7% resistivity in all organisms (details in Table 2). 33.08% of the isolated gram negative bacteria were found to be multidrug resistant. MDR were classified as bacteria resistant to two or more antibiotics.

Although the frequency of organisms varies significantly, highest number of MDR was found to be in *E. coli* (43.14%) followed by *Pseudomonas* (28.57%), *Acinetobacter* (26.31%), *Klebsiella* (25.71%) which are details in Table 3.

Table 2. Antibiotic resistance number of gram negative isolates.

Group	Antibiotics	Organisms			
		E.coli	*Klebsiella*	*Pseudomonas*	*Acinetobacter*
Fluoroquilones	Ciprofloxacin	23	10	7	5
	Nalidixic Acid	13	2	2	0
	Ofloxacin	12	8	2	0
	Norfloxacin	22	8	1	5
Aminoglycosides	Amikacin	7	5	3	1
Tetracycline	Tetracycline	5	6	1	0
Penicillins	Amoxycillin	21	3	2	1
	Ampicillin	9	0	0	0
	Cloxacillin	13	9	9	2
Sulphonomides	Cotrimoxazole	15	14	5	3
Cephalosporins	Cephalexin	2	2	1	0
	Ceftriazone	2	1	1	0
Miscellaneous	Chloramphenicol	12	1	0	2

Table 3. Occurrence of multi drug resistant organisms.

Organisms	Total Isolates	MDR Isolates n (%)
E.coli	51	22 (43.14)
Klebsiella	35	9 (25.71)
Pseudomonas	28	8 (28.57)
Acinetobacter	19	5 (26.31)
Total	133	44 (33.08)

Discussion

This study provides the evidence of high prevalence of antibiotic resistant bacteria in the samples of patients collected from a tertiary care hospital environment. The resistant rate of gram negative pathogens to different tested antibacterial agents was considerably high and has increased during the recent years. Our findings indicate the predominance of *E. coli* among the bacterial isolates of the samples.

This is in accordance with a European and North American surveillance study on more than 220000 samples collected from intensive care units during 2000-2002 (Jones *et al.,* 2004). A cross-sectional study on 86 patients with infected diabetic foot admitted to Nemazee hospital from July 2011 to June 2012 demonstrated *E. coli* as the most common gram-negative bacteria (Anvarinejad *et al.*, 2015). Similar findings were also reported by (Khalili *et al.,* 2012) and (Moradi *et al.,* 2015) from 2 teaching hospitals at Tehran and Bandar Abbas in Iran, respectively.

The prevalence and antibiotics resistance patterns of pyogenic bacterial isolates usually exhibit variability according to geographic areas and climate conditions. Existence of high drug resistance to multiple antibiotics in *E. coli*, *Klebsiella*, *Pseudomonas* and *Acinetobacter* isolates from the isolates including blood, urine, sputum, wound swab and stool samples, indicates the negligence on patients part, incomplete treatment schedules, antibiotics misuse, self-prescription, misprescription, lack of regional antibiogram data, and limited knowledge about multidrug-resistant isolates and antimicrobial resistance among clinicians. Updated knowledge of antimicrobial susceptibility profiles of clinical isolates will not only assist in designing the most appropriate dose-regimen and treatment schedule against wound infections but also help in curbing the alarmingly expanding menace of drug resistance.

Gram-negative infections were responsible for more severe infections and case fatality (Ghosh, 2009). Severity of the cases increased by drug-resistant pathogens in hospitalized patients with serious infections such as pneumonia, urinary tract infections (UTI), skin and skin-structure infections, and primary or secondary bacteremia which is generally ascribed to the widespread use of antimicrobial agents (Jones, 1996).

In a recent report, the Infectious Diseases Society of America specifically addressed three categories of MDR gram-negative bacilli, namely, extended-spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella spp.,* MDR *Pseudomonas aeruginosa*, and carbapenem-resistant *Acinetobacter spp.*(Falagas *et al.,* 2005). Moreover, there are now a growing number of reports of cases of infections caused by gram-negative organisms for which no adequate therapeutic options exist (Talbot *et al.,* 2006). This return to the pre-antibiotic era has become a reality in many parts of the world (Coelho *et al.,* 2004; Paterson and Bonomo, 2005; Walsh *et al.,* 2005).

Among the species *E. coli* and K. *pneumoniae*, a worrisome trend during the last two decades has been the development of resistance to extended spectrum cephalosporins, e.g., cefotaxime, ceftazidime, and ceftriaxone (Paterson, 2006).

Our findings implicated that resistant rate of gram-negative pathogens to different antibacterial agents, especially fluoroquilones are considerably high and increased during the recent years. *E. coli* is associated with the highest rate of resistance among the isolates followed by *Pseudomonas*, *Acinetobacter* and *Klebsiella*. Performing regular and periodic surveillance of antimicrobial resistance pattern by the comprehensive, multi-specialty infection control committee appear to have a key role in optimizing both the empirical and definite antibacterial treatment regimens and may also slow down the runaway train of antibacterial resistance at our clinical settings in India.

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