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ORIGINAL ARTICLE

Antibiotic Susceptibility Pattern of Gram Negative Bacilli Isolated from Different Clinical Specimens in a Tertiary Care Hospital

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ABSTRACT

Objective: To find out the antibiotic susceptibility pattern of gram-negative bacilli isolated from different clinical specimens received in a tertiary care hospital at Wah.

Material and Methods: This cross-sectional study was carried out to determine antibiotic susceptibility pattern of gram-negative bacilli, cultured from different clinical specimens received in POF Hospital laboratory at Wah. One hundred and forty-four clinical isolates of gram-negative rods from different clinical specimens from April 2015 to March 2016 were included in the study. All the isolates were processed by standard microbiological methods. The antibiotic susceptibility pattern was carried out by disk diffusion method as recommended by Clinical Laboratory Standard Institute guidelines (CLSI).

Results: Out of one hundred and forty-four Gram-negative bacilli, one hundred (69.44%) were from *Enterobacteriaceae* family and forty-four (30.56%) were from *non-Enterobacteriaceae group.* The commonest isolated organism was *Escherichia coli* (47.3%), followed by *Pseudomonas aeruginosa* (17.36%) and *Acinetobacter baumannii* (13.19%). These isolates were highly resistant to the most of the commonly prescribed antibiotics. The members of the family *Enterobacteriaceae* showed better sensitivity for amikacin and cefoperazone-sulbactam. Resistance rate for carbapenems was significantly high for *K.pneumoniae* and *Proteus mirabilis*. Among *non-Enterobacteriaceae*, *P.aeruginosa* showed better susceptibility for cefoperazone-sulbactam, amikacin, imipenem and meropenem. The multi-drug resistant pattern was observed for *Acinetobacter.baumannii.*

Conclusion: The isolates depict highly resistant patterns to available oral antibiotics as well as commonly prescribed injectable third generation cephalosporins and carbapenems. Establishment and implementation of infection control practices are required to combat this grave situation.

Key words: Antibiotics, Antibiotic susceptibility, Enterobacteriaceae, Gram negative bacilli, Non-Enterobacteriaceae.

Author`s Contribution
[1] Conception, synthesis, planning of research and manuscript writing Interpretation and discussion
[2] Data analysis, interpretation and manuscript writing, [3] Active participation in data collection.

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Introduction

Antibiotics have enabled tremendous advances in the discipline of infectious diseases since their emergence in 1930. Unfortunately, the occurrence of resistant bacteria is endangering the efficacy of antibiotics, which have transformed medicine and saved millions of lives.[1] The infections caused by multiple-drug resistant (MDR) gram-negative organisms have created entire classes of antibiotics redundant and threatened to bring about the end of the 'antibiotic era'. [2,3] Extended-spectrum beta-lactamases, Metallo-beta-lactamases and Amp-C

mediated beta-lactamases produced by *Enterobacteriaceae* and other non-lactose fermenters are increasingly implicated in outbreaks through the dissemination of mobile genetic elements rendering emergence of resistant mutants.[4] This grave situation demands optimization of therapy primarily because of substantial increases in the frequency with which these organisms affect the health care settings as well as the community acquired infections. The challenge of heightened antimicrobial resistance among gram-negative pathogens has been exacerbated by the stagnation in the development of novel antimicrobials. Studies have provided convincing evidence that effective initial empirical antibiotic therapy, based on ultimate drug susceptibility results, improves survival.[2]

The constantly evolving antimicrobial resistance patterns render antibiotic susceptibility profile in one region at a specific period, inapplicable to other region or in another period. Thus, antimicrobial susceptibility data from any given regional, national, or international surveillance study cannot reliably predict the drug resistance profiles of pathogens isolated from an individual patient.[5] The current study was designed to document the commonly isolated gram-negative bacilli from different clinical specimens and their susceptibility patterns in tertiary health care hospital at Wah. This will be an effort to rationalize the empirical treatment by clinicians resulting in evidence-based practice and better results in terms of early recovery from infections, shorter duration of hospitalization and cost effectiveness. Moreover, this effort will contribute to safeguard the remaining therapeutic options for the clinicians and encourage a focused, concerted effort against key human pathogens.

Material and Methods

This cross-sectional study was carried out at Microbiology section of Pakistan Ordinance Factories Hospital laboratory from April 2015 to March 2016. One hundred and forty-four clinical specimens from patients either gender, of all ages, yielding growth of Gram-negative bacilli were included in the study and selected by convenient sampling. Duplicate samples of the same patient from the same site were not included.The specimens were inoculated on appropriate culture medium like blood agar, MacConkey agar, chocolate agar (sputum) and cysteine lactose electrolyte deficient agar (urine) and incubated at 35-37˚C under aerobic conditions for 24 hours. After overnight incubation, the agar plates were examined for growth of bacteria and their colonial morphology. Gram-negative rods were identified based on Gram staining, catalase test, oxidase test and motility.[6] Microbact Gram-negative 24E identification kits (Oxoid, Basingstoke, UK) were used for confirmation of isolates. Antimicrobial susceptibility tests were performed on the Muller–Hinton agar plates with disk diffusion method, as recommended by clinical laboratory standards institute.[7]

The bacterial suspensions of isolates equivalent to 0.5 McFarland standard turbidity were applied on Mueller-Hinton agar (Oxoid, Basingstoke, UK). The antimicrobial disks (Oxoid, Basingstoke, UK) were evenly placed on the inoculated plates and included Ampicillin (10 µg), amoxicillin-clavulanate (20/10 µg), trimethoprin-sulfamethoxazole (1.25/ 23.75 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), cefoperazone-sulbactam (75/ 30 µg), imipenem (10 µg) and meropenem (10 µg). Concurrent quality control testing was performed with *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. After overnight incubation, the diameter of each zone of inhibition around the antimicrobial disk was measured. The susceptibility results were interpreted according to recommendations of CLSI as sensitive, intermediate and resistant.[7] The data were entered and analyzed using SPSS version 19. For qualitative variables (Gram-negative bacilli, gender, type of samples and organisms isolated) frequencies and percentages were calculated. Mean ± SD was presented for age.

Results

A total of one hundred and forty-four (144) isolates of Gram-negative bacilli were included in the study. Majority of isolates were yielded from urine (n=83, 57.6%), followed by pus (n=23, 16%) and respiratory specimens (n=17, 11.9%). The other isolates were from blood, high vaginal swabs, catheter tips, ear swabs, tissue and body fluids. The distribution of specimens along with their breakup is presented in table 1. Out of one hundred and forty-four (144) Gram-negative bacilli, one hundred (69.44%) were members of the family *Enterobacteriaceae*

Table 1: Break up of clinical specimens yielding gram-negative bacilli (n=144)		
	Frequency	Percentage
Blood	8	5.6
Tracheostomy discharge	8	5.6
Endotracheal tubes	6	4.2
CVP	1	.7
CSF	1	.7
Vitreous tap	1	.7
Bile	1	.7
Tissue	1	.7
Bronchoalveolar lavage	1	.7
Peritoneal fluid	2	1.4
Urine	83	57.6
High vaginal Swabs	1	.7
Sputum	2	1.4
Catheter tips	4	2.8
Pus	23	16.0
Ear swab	1	.7

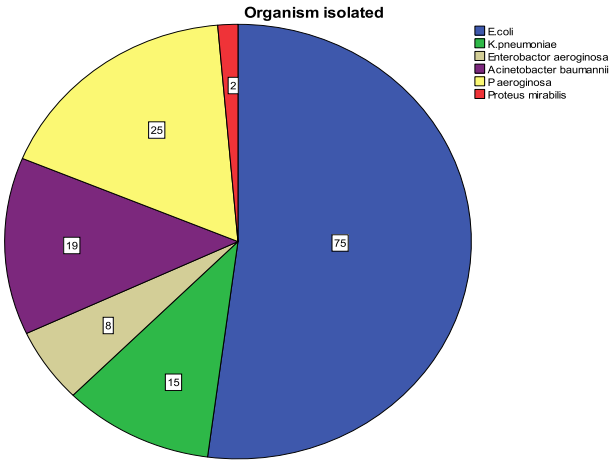

Figure 1: Frequency of isolated gram-negative bacilli (n=144)

and forty-four (30.56%) were from non-*Enterobacteriaceae*. and forty-four (30.56%) were from non-*Enterobacteriaceae*.

The commonest isolated organism was *Escherichia coli* (47.3%), followed by *Pseudomonas aeruginosa* (17.36%) and *Acinetobacterbaumannii* (13.19%). (Figure-1) Out of one hundred and forty-four isolates, 53.47% were recovered from male patients and 46.53% from female patients. Mean age of the patients was 49.32years± 23.72 SD. Age distribution of different age groups which yielded Gram-negative isolates is shown in Figure-2.

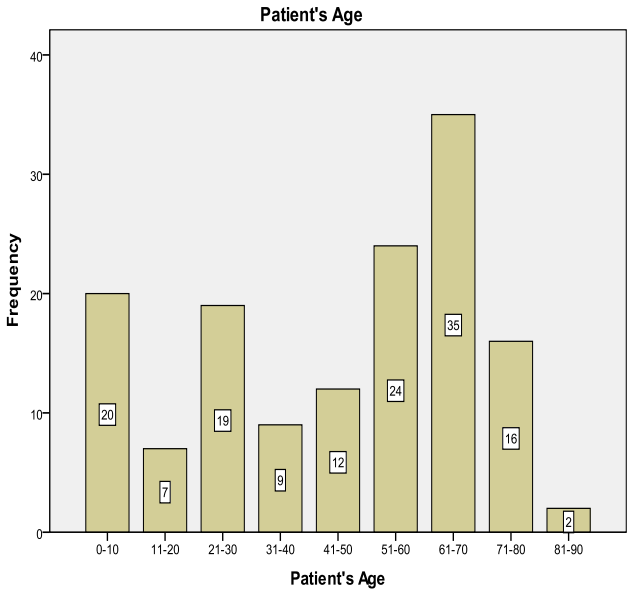

Figure 2: Distribution of different age groups, which yielded gram- negative bacilli

Thirty-seven isolates (33.3%) were isolated from outdoor patients, while the remaining (n=107, 66.7%) were from patients admitted in different wards. Their distribution is presented in Figure 3.

The resistance frequency of *E.coli* against ampicillin, amoxicillin-clavulanate and trimethoprim-sulfamethoxazole was 93.3%, 81.3% and 76% respectively. None of the isolates was 100% susceptible to any of the antimicrobials assessed in the study.

Table 2: Antimicrobial resistance pattern in Enterobacteriaceae								
	Escherichia coli (n=75)		Klebsiella pneumonia (n=15)		Enterobacter Spp (n=08)		Proteus mirabilis (n=02)	
	n	R%	n	R%	n	R%	n	R%
AMP	70	93.3	15	100	7	87.5	2	100
AMC	61	81.3	14	93.3	7	87.5	2	100
AK	14	18.6	7	46.6	3	37.5	2	100
G	32	42.6	8	53.3	3	37.5	1	50
COT	57	76	11	73.3	6	75	2	100
CRO	51	68	11	73.3	4	50	1	50
IMP	17	22.6	8	53.3	1	12.5	1	50
MNP	21	28	8	53.3	2	25	1	50
SCF	10	13.3	5	33.3	2	25	1	50
CIP	51	68	8	53.3	3	37.5	1	50

The antibiotic susceptibility pattern of members of *Enterobacteriaceae*, including *E.coli, K.pneumoniae, Enterobacter sp* and *Proteus mirabilis* has been shown in Table 2.

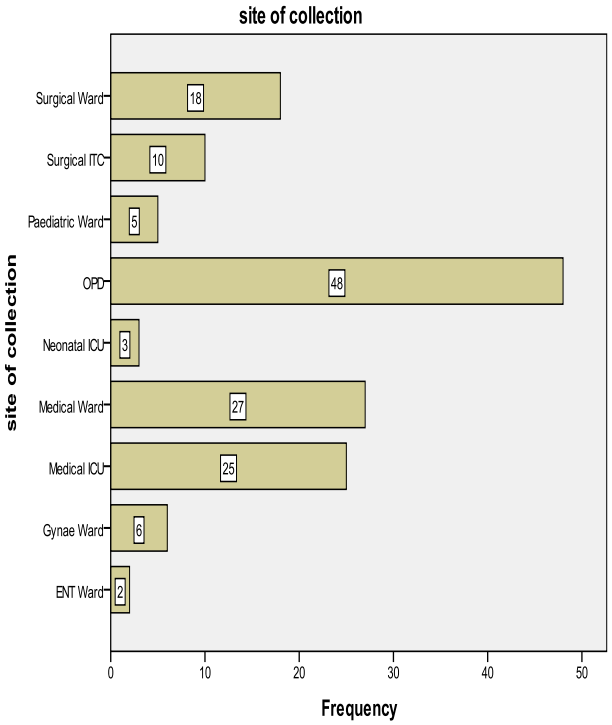

Figure-3: Sites of collection of gram-negative bacilli.

The susceptibility pattern of members of *non-Enterobacteriaceae* including *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is displayed in Table 3.

Table 3: Antimicrobial resistance pattern in *Non-Enterobacteriaceae*				
	Pseudomonas Aeruginosa (n=25)		Acinetobacter Baumannii (n=19)	
	n	R%	n	R%
AMP	_	_	19	100
AMC	_	_	19	100
AK	13	52	15	78.9
G	20	80	17	89.4
COT	_	_	18	94.7
CRO	_	_	17	89.4
CAZ	24	96	_	_
IMP	13	52	18	94.7
MNP	13	52	18	94.7
CIP	18	72	17	89.4
SCF	12	48	12	63.1
DOX	_	_	13	68.4

AMP-Ampicillin, AMC-Amoxicillin-clavulanate, AK-Amikacin, G-Gentamicin, COT-Trimethoprim-sulfamethoxazole , CRO- Ceftriaxone, CAZ- Ceftazidime, IMP- Imipenem , MNP- Meropenem, SCF- Cefoperazone-sulbactam , CIP- Ciprofloxacin ,DOX- Doxycycline, R-Resistant

Discussion

The susceptibility pattern of Gram-negative isolates revealed an alarming resistance ratio to commonly used antibiotics. In the present study, *E.coli*, *Klebsiella pneumoniae*, *Enterobacter spp* and *Proteus mirabilis* are highly resistant to first-line drugs including ampicillin, trimethoprim-sulfamethoxazole and amoxicillin-clavulanate. These findings are in agreement with a study carried out at Fauji Foundation Hospital Rawalpindi by Nabi et al.[8] It is worrisome to note the high rates of resistance of members of *Enterobacteriaceae* to the third generation cephalosporins (ceftriaxone) and also to the commonly used flouroquinolone (ciprofloxacin) in our study. Similar resistance patterns have been reported in other studies from Rawalpindi[4] as well as from Iran.[9] This situation is much different when compared to the resistance rate prevailing in England as published in English surveillance programme for antimicrobial utilization and resistance (ESPAUR) report, 2014.[10] The contrasting results provide an evidence of injudicious and imprudent use of these antibiotics in our setup.

The resistance frequency of *E.coli* against gentamicin (42.67%) and amikacin(18.67%) is comparable to a study conducted on uropathogens by Nabi et al in Dhaka in 2014.[11] A low percentage of resistance of *E.coli* against amikacin indicate that this antibiotic may be a useful therapeutic agent in our setup when considered as empirical choice. *Klebsiella pneumoniae is* fairly resistant to gentamicin and amikacin. This finding is in concordance with those reported by Bhat et al.[12] A high and moderate level of resistance of *Proteus mirabilis* against amikacin and gentamicin respectively are depicted in our study, a situation different from the one reported by Bahashwan et al.[13] A disturbing situation existing in our hospital is 53.3% resistance against imipenem and meropenem conferred by *K.pneumoniae*, and 50% for both members of carbapenems by *P.mirabilis*. Similar results have been reported from other parts of the subcontinent.[11,13] Poor infection control measures contribute to the development of high-level resistance to these relatively safe and effective antibiotics. Resistance of *E.coli* and *Enterobacter spp* against carbapenems are also significantly high when compared to studies conducted in two different institutions at Rawalpindi.[4,8] Our study also revealed *A.baumannii* is significantly resistant (89.4%) to each of ceftriaxone, gentamicin and ciprofloxacin. This bug shows 94.4% resistance to carbapenems and trimethoprim-sulfamethoxazole. The resistance pattern in our settings is in accordance when compared to data by Fayyaz et al[16] in Rawalpindi and Sohail et al in Lahore.[17] Cefoperazone-sulbactam and doxycycline are relatively effective drugs against *A.baumannii* (63.1% & 68.4% respectively) in our setting , which is a similar finding as demonstrated by Fayyaz et al.[16] Our data suggests 96% resistance of *P.aeruginosa* against ceftazidime, 72% against ciprofloxacin and 52% against amikacin , imipenem and meropenem respectively. Comparison with ESPAUR report revealed a stupendous difference in susceptibility pattern of *P.aeruginosa*, indicative of a failure of antibiotic stewardship in our settings.[10] Antibiograms in context to local data unveiled an increased resistance ratio to antipseudomonal antibiotics as compared to our clinical settings.[4,18] Our study is laboratory-based and has no correlation with the clinical outcomes after antibiotics administration to treat the specific pathogen. Despite this limitation, the study will be helpful for local clinicians to make an appropriate choice of antibiotic for empirical therapy.

Conclusion

E.coli is the most common Gram-negative bacillus, followed by *P.aeruginosa* and *A.baumannii* isolated from the culture of clinical specimens in POF Hospital, Wah. The isolates depict highly resistant patterns to available oral antibiotics as well as commonly prescribed injectable third generation cephalosporins and carbapenems.

1. Gram-negative bacilli reveal relatively better susceptibility against Cefoperazone-sulbactam.
2. Antibiotic resistance is a dynamic phenomenon which emphasizes continuous monitoring of infection control practices and regular surveillance of antibiotics susceptibility patterns in our health care setting.
3. Strict implementation of polices for judicious use of antibiotic and efficient infection control practices are strongly recommended.

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