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Evaluation of Antimicrobial Susceptibility of Gram Negative Organisms by Minimum Inhibitory Concentration Method in Bandar Abbas, South of Iran

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ABSTRACT

Background: Antibiotic-resistant pathogens are problem in many geographic areas. The selection of appropriate antibiotics to treat Gram-negative bacteremia may be life-saving. The aim of this study was to evaluate the incidence of decreased antibiotic susceptibility among aerobic gram-negative bacilli isolated from hospitalized patients of Bandar Abbas general hospital.
***Methods*:** Consecutive specimens collected on clinical indications from hospitalized patients were cultured. The minimum inhibitory concentrations (MICs) of antimicrobial agents were determined by the agar dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines.
***Results*:** In this study 494 positive samples during one year were evaluated. The most common site of isolation was urinary system. The most frequently isolated organisms were *E.coli* (%47.3), *Kelebsiella pneumonia* (%19.9) and *Pseudomonas aeruginosa* (%16.2). Resistance to ceftriaxone, ceftazidime and cefepime was more than %50. MIC results revealed that E.coli was sensitive to amikacin, ciprofloxacin and meropenem more than %75; whereas sensitivity to ceftriaxone, ceftazidime and cefepime was less than %30. Klebsiella showed sensitivity to amikacin %77.1; however sensitivity to ceftriaxone, ceftazidime and cefepime less than %25. In addition, sensitivity of *Pseudomonas aeruginosa* to amikacin, ciprofloxacin and imipenem was more than %65; while sensitivity to ceftriaxone, ceftazidime and cefepime was %25 or less.
Conclusions: The high incidence of reduced antibiotic susceptibility among gram-negative bacteria in this hospital suggests that more effective strategies are needed to control the selection and spread of resistant organisms.

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Introduction

Resistance to higher antimicrobial agent is commonly seen in gram negative bacilli. This issue is a challenging problem to the medical practitioners in addition to it is financial impact on the health care system (1). Treatment of serious life threatening infections due to multi-drug resistant pathogens presents a difficult challenge due to the limited therapeutic options (2). The rapid emergence of resistant bacteria is the result of different factors as the

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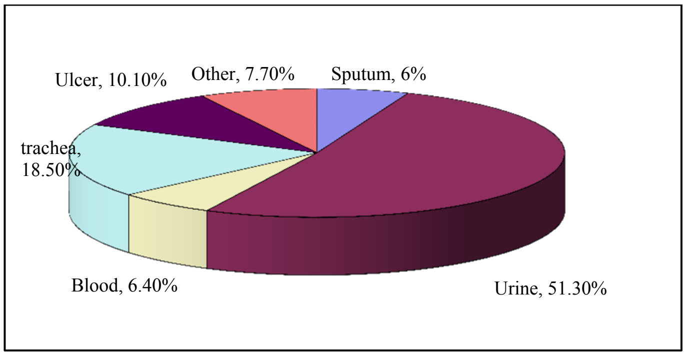

Figure 1. Frequency of isolated samples.

intrinsic microbial complexity, the growing attitude to travel of humans, animals and goods, the use of antibiotics outside hospitals, and the lack of precise therapeutic chooses for high risk group of patients (3, 4). The aim of this study was to evaluate the incidence of decreased antibiotic susceptibility among aerobic gram-negative bacilli isolated from hospitalized patients of Bandar Abbas general hospital.

Methods

In a cross sectional prospective study during one year between 2014 to 2015, clinical samples including urine, sputum, vascular catheter, cerebrospinal fluid, wound, trachea, pharyngeal, ear and ophthalmic discharge were cultured and evaluated in Bandar Abbas general hospital, south of Iran. Samples were obtained from outpatient and inpatients. Samples were cultured on appropriate media like blood agar and Mac Cankey agar. Organisms were identified by microbiologic methods. The Minimum inhibitory concentrations (MICs) of antimicrobial for seven antibiotics including: amikacin, ceftazidime, ciprofloxacin, ceftriaxone, imipenem, meropenem and cefepime were determined by the agar dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines. MIC is defined as the lowest concentration of antimicrobial agent required to inhibit growth of the bacteria. The break points for susceptible and resistant categories were as follows: imipenem, 4 mg/L or less and 16 mg/L or more; ceftazidime, 8 mg/L or less and 32 mg/L or more; ceftriaxone, 8 mg/L or less and 64 mg/L or more; piperacillin, 16 mg/L or less and 128 mg/L or more (except *P aeruginosa,* ≤64 mg/L and ≥128 mg/L); piperacillin-tazobactam, 16/4 mg/L or less and 128/4 mg/L or more (except *P aeruginosa,* ≤64/4 mg/L and ≥128/4 mg/L); gentamicin, 4 mg/L or less and 16 mg/L or more; amikacin, 16 mg/L or less and 64 mg/L or more; and ciprofloxacin, 1 mg/L or less and 4 mg/L or more. Table 1 shows MIC used for each antibiotic. For the agar dilution method, *E.coli*, *Kelebsiella pneumonia* and *Pseudomonas aeruginosa* suspensions were adjusted to the turbidity equivalent to a 0.5 McFarland standard, approximately 104 CFU of these suspensions was inoculated onto Mueller-Hinton agar containing a twofold dilution series of antibiotics and supplemented with 5%

Table 1. Concentration for antibiotics in MIC.

MIC (µg/ml)	Antibiotics
16-32	Amikacin
8-16-32	Ceftazidime
1-2-4	Ciprofloxacin
8-16-32-64	Ceftriaxone
4-8-16	Imipenem
4-8-16	Meropenem
8-16-32	Cefepime

MIC: minimum inhibitory concentration.

Table 2. Susceptibility rates of isolated microorganism.

Organism	Samples	Antibiotic NCCLS breakpoint MIC value (μg/ml) Amikacin 16			Ceftazidime 8			Ciprofloxacin 1			Ceftriaxone 8			Imipenem 4			Meropenem 4			Cefepime 8		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ecoli	Inpatient (%)	71.2	-	28.8	15.2	21.7	63.1	61.1	14.8	24.1	20	33.3	46.7	67.7	3.2	29.1	77.5	10	12.5	22.6	12.9	64.5
	Outpatient (%)	85	-	15	33.3	20.3	46.4	85.1	3.5	11.4	28.4	40.7	30.9	70.7	6.5	22.8	79.4	6.6	14	23.2	8.9	67.9
Klebsiella	Total (%)	-	20.9	26.9	20.8	52.3	75.9	7.8	16.3	25.4	38.1	36.5	69.5	5.2	25.3	78.6	8	13.3	23	10.3	66.7	
	Inpatient (%)	73.5	-	26.5	12.1	15.2	72.7	50	25	25	21.9	18.7	59.4	45.7	10.8	43.5	58.9	19.7	21.4	4.2	8.3	87.5
	Outpatient (%)	85.7	-	14.2	26.3	15.8	57.9	59.1	13.7	27.2	17.6	41.2	41.2	62.5	12.5	25	50	22.7	27.3	25	18.7	56.3
Pseudomonas	Total (%)	-	22.9	17.3	15.4	67.3	53.2	21	25.8	20.4	26.6	53.1	51.4	11.4	37.2	56.4	20.5	23.1	12.5	12.5	75	
	Inpatient (%)	59.3	-	40.7	30.4	0	69.6	64.5	12.9	22.6	0	11.7	88.3	69.7	6.1	24.2	40.5	19	40.5	12.5	6.2	81.3
	Outpatient (%)	100	-	0	0	20	80	71.4	0	28.6	33.3	16.7	50	50	20	30	50	12.5	37.5	0	0	100
	Total (%)	-	34.4	25	3.6	71.4	65.8	10.5	23.7	8.7	13	78.3	65.1	9.3	25.6	42.2	17.8	40	10.5	5.3	84.2	

S: Sensitive, I: Intermediate, R: Resistance

MIC: minimum inhibitory concentration, NCCLS: National Committee for Clinical Laboratory Standards

defibrinated sheep blood using a multipoint inoculator (a Cathra replicator system) with 1-mm pins (Oxoid, Inc., Ogdensburg, NY). Data was transferred to SPSS® 19 Software for statistical analysis. The nominal variables were stated number and percentages.

Results

In this study 494 positive cultured samples from patients with documented infection were evaluated. It includes inpatient (%59.3) and outpatient (% 40.7) samples. %41.5 of samples were collected from women and %58.5 from men. The most wards that samples were obtained included internal Intensive Care Unit (ICU), surgical ICU and internal wards respectively. The most common site of isolation was urinary system (%51.3) (Figure 1). The most common isolated organisms were *E.coli* (%47.3), *Kelebsiella pneumonia* (%19.9) and *Pseudomonas aeruginosa* (%16.2).

MIC data were stated in Table 1 and 2. Our study showed that E.coli showed sensitivity to amikacin, ciprofloxacin and meropenem more than %75. E.coli showed sensitivity to ceftriaxone, ceftazidime and cefepime less than %30. Sensitivity to imipenem was %69.5.

Kelebsiella pneumonia showed sensitivity to amikacin (%77.1). *Kelebsiella pneumonia* showed sensitivity to ceftriaxone, ceftazidime and cefepime less than %25. Sensitivity to ciprofloxacin, imipenem and meropenem was about %50.

Pseudomonas aeruginosa showed sensitivity to amikacin, ciprofloxacin and imipenem more than %65. Sensitivity to ceftriaxone, ceftazidime and cefepime was %25 or less. Sensitivity to meropenem was %42.2.

Analysis of data about inpatient and outpatient samples (sensitivity, intermediate resistance and resistance) was shown in Table 2.

Discussion

Over the last decade the proliferation of antibiotic-resistant pathogens has been a growing problem, especially in some geographic areas, making useless most of the classical antibiotic therapies. In Patel's study out of total 328 isolates, 118 (35.98%) were *E.coli*, 72 (21.95 %) *Kelebsiella pneumonia*, 64 (19.51%) *Pseudomonas aeruginosa*, 30 (9.15%) *Acinetobacter baumannii*, 18 (5.49%) *Proteus vulgaris*, 18 (5.49%) *Proteus mirabilis*, 6 (1.83%) *Providencia rettgerii*, and 2 (0.61%) *Citrobacter freundii*. Out of these isolates, 228 (69.51%) were β-lactamase positive (1). In Akhtar's study in India the isolation rate of Gram-positive bacteria was relatively low. Majority (> 50%) of the Gram-negative isolates were resistant to many of the antibiotics tested. Relatively low resistance was only observed against amikacin (21.3%) and imipenem (26.1%). Majority (> 60%) of Gram-negative isolates were resistant to cefotaxime, ceftriaxone and ceftazidime. The isolates showed high resistance to ofloxacin (65.9%) and ciprofloxacin (73.9%) (5). In another study among the gram-negative rods, fewer than 10% were resistant to imipenem, piperacillin-tazobactam, amikacin, gentamicin, ceftazidime, cefepime, and tobramycin, whereas more than 80% showed resistance to ampicillin and cefazolin (6). In Somily's study using the MIC method, colistin was found to be active against 100% of *Acinetobacter* species, 98% of *A. baumannii*, 84% of *P. aeruginosa*, and 79% of *S. maltophilia* were sensitive to colistin. An ascending order MIC90 of colistin was 1 microg/ml for *A. baumannii*, 1.5 microg/ml for *Acinetobacter species*, 3 microg/ml for *P. aeruginosa*, and 16 microg/ml for *S. maltophilia*. Comparing disk diffusion method with the Etest method, very major errors of 1.4% were found for *A. baumannii* and 2.3% for *P. aeruginosa*, with minor errors of 0.7% for *A. baumannii*, 8.3% for *S. maltophilia*, and 11.6% for P. *aeruginosa* (7).

Antibiotic resistant pattern in urinary tract infection showed that E. coli had higher susceptibility to ceftazidime (87.4%), cefuroxime (85.1%) and ceftriaxone (76.6%) than to sulfamethoxazole (SMZ) (8.0%), amoxicillin (21.7%), ampicillin (17.1%) and cefazolin (37.7%). Isolates of *Klebsiella pneumonia* and Proteus species had similar patterns as *E.coli* (8). In a study on uropathogen in South Africa the most common isolate was *E.coli* (39.0%) followed by Klebsiella species (20.8%) and *Enterococcus faecalis* (8.2%). The Gram-negative isolates displayed a very high level of resistance to amoxicillin (range 43-100%) and co-trimoxazole (range 29-90%), whereas resistance to gentamicin (range 0-50%) and ciprofloxacin (range 0-33%) was lower. E. coli isolates were susceptible to nitrofurantoin (94%), and Extended-spectrum beta-lactamases (ESBL) production was significantly higher (P: 0.01) in the hospital isolates, compared with those from the community referral sites (9).

Antimicrobial resistance is an emerging problem. Justifying new more stringent antibiotic prescription guidelines, Continuous monitoring of antimicrobial susceptibility and strict adherence to infection prevention guidelines are essential to eliminate major outbreaks in the future (10). The rapidly escalating prevalence of antimicrobial resistance is a global concern. This reduced susceptibility to currently available antimicrobial agents coupled with the progressive shortage of newly approved compounds is a worrisome situation (11). The E-test is a useful tool for determining MICs and testing antimicrobial combinations (12).

In conclusion, the high incidence of reduced antibiotic susceptibility among gram-negative bacteria in this hospital suggests that more effective strategies are needed to control the selection and spread of resistant organisms.

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