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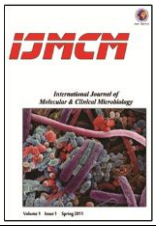

Survey of antibiotic susceptibility pattern in microorganisms isolated from clinical samples in Shahid Faghihi hospital of Shiraz, Iran

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ABSTRACT

Antibiotic resistance is a public concern, worldwide. The purpose of this study was to survey the antibiotic susceptibility pattern in the microorganisms isolated from the clinical samples in Shahid Faghihi hospital of Shiraz, Iran. In this study, the microorganisms were isolated from the blood, bone marrow, pleural fluid, CSF and Vaginal discharge. Antibiotic susceptibility test was performed by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) standards. Out of 1344 samples, 306 (22.7%) various organisms were obtained. The highest and lowest isolates were related to blood (94.8%) and bone marrow (1.2%), respectively. Among all isolates, *Staphylococcus aureus* (32.6%) was the most common bacterium. *Escherichia coli* with 20.9% and *Staphylococcus aureus* with 64.9% were the most common Gram-negative and Gram-positive isolated bacteria, respectively. Antibiotic susctibility test results also showed that the highest and lowest resistances were related to *Acinetobacter* and *Salmonella*, respectively. Awareness of the antibiotic resistance can lead to proper administration of antibiotics in treating of bacterial infections and preventing antibiotic resistance.

1. Introduction

Antibiotics are important as antimicrobial agents for treatment of infectious diseases (Rachel and Hillary, 2014). In the recent years, antibiotic resistance has increased widely, which has led to an extensive defeat in antibiotic therapy, particularly in intensive care units (ICUs) (Martinez, 2014). Misuse, abuse and overuse of antibiotics are the main reasons to appear resistant bacteria in clinic (English BK and Gaur, 2010). In some countries, arbitrary use of antibiotics and food additives in poultry and household cleaners can cause mutations and selection pressure in the microorganism that lead to the creation of resistant strains. Methicillin-resistant *Staphylococcus aureus* (MRSA) is probably the most well-known, but others, including VISA (vancomycin-intermediate *S.aureus*), VRSA (vancomycin-resistant *S.aureus*), VRE (Vancomycin-resistant *Enterococcus*), MRAB

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(Multidrug-resistant *A.baumannii*) and KPC (*Klebsiella pneumoniae* carbapenemase) are prominent examples too (Bonnie et al., 2012; Patrick et al., 2003; SLee et al., 2012).

Antibiotic resistance in microorganism populations can occur both genetically and environmentally. Genetic resistance spreads both "vertically," when inherit antibiotic resistance genes, and "horizontally," when bacteria transfer genetic material to other bacteria. Horizontal gene transfer can happen between various bacterial species. Environmentally, antibiotic resistance spreads occur when bacteria are transferred from place to place via airplanes, water and wind.

Resistant bacteria can also be transferred from one to one by coughing, sneezing and direct contact (Howard et al., 2005). Data from Study for Monitoring Antimicrobial Resistance Trends (SMART) show that difference in the level of antimicrobial resistance in the various geographic regions is related to hygiene, travel, conflict, trade, and disease (Stephen et al., 2009). Delay in treatment of infectious diseases could lead to increase the mortality and morbidity in patients with severe infections, particularly those caused by antibiotic resistant bacteria (Sader et al., 2013). In Europe, it is estimated that about 25,000 people die of antibiotic-resistant bacterial infections annually (Otto et al., 2011). In 2005, about 94,000 hospitalization patients were infected by MRSA in the USA, of which 19,000 ended to death (Hanson et al., 2011). A new report by the Centers for Disease Control and Prevention (CDC) has estimated that at least two million diseases and 23,000 deaths occur by the antibiotic resistant bacteria annually in the USA (Brett et al., 2014). First Warning Systems (FWS) are necessary to diagnose and warn the antibiotic resistance patterns in local, regional, national and global level. In addition, antibiotic susceptibility pattern is essential for treatment of the infections and prevention of antibiotic resistance. The aim of this study was to survey the antibiotic susceptibility pattern in microorganisms isolated from the clinical samples in Shahid Faghihi hospital of Shiraz in Iran.

2. Material and Methods

2.1. Clinical specimens and laboratory identification

A cross - sectional study was conducted during a one year period of time effective from April till March, 2013, in Shahid Faghihi hospital in Shiraz, Iran. Clinical samples including blood, bone marrow, pleural effusion, vaginal discharge and cerebrospinal fluid (CSF) were collected from the hospitalized patients. Collected samples were added to the brain-heart infusion broth medium (Merck, Germany) and transferred to the microbiology laboratory of Shahid Faghihi hospital in Shiraz, Iran. Samples were cultured on the blood agar, chocolate agar, MacConkey agar and thioglycollate broth (Merck, Germany) and plates were incubated at 37°C for 24-48h but blood and bone marrow samples in order to enrichment were inoculated to a biphasic media for one, two and three week, after this time the samples were streaked culture on blood agar and chocolate agar (Merck, Germany). Then, all suspected colonies were identified by the standard biochemical and microbiological tests.

2.2. Antimicrobial susceptibility testing

Antibiotic susceptibility test was performed by disc diffusion method on Mueller Hinton agar, as recommended by the Clinical and Laboratory Standards Institute (CLSI) (Wayne PA, CLSI; 2010). Antimicrobial susceptibility test was done by 26 antibiotics (Mast, Germany) including rifampin (5µg), cephalothin (30µg), vancomycin (30µg), penicillin (10µg), ampicillin (10µg), amoxicillin (10µg), erythromycin (15µg), clindamycin (2µg), ciprofloxacin (5µg), cefoxitin (30µg) , cefazolin (30µg), gentamicin (10µg), tetracycline (30µg), co-trimoxazole (25µg), methicillin (5µg), cephalexin (30µg), imipenem (10µg), amikacin (30µg), cefepime (30µg), ceftriaxone (30µg), sulbactam (30µg), tazobactam-piperacillin (10/100µg), ceftazidime (30µg), ofloxacin (5µg), streptomycin (10µg) and fluconazole (25µg). *Escherichia coli* ATCC 25922 was used as quality control strain.

3. Results

Of 1344 collected samples, 630 and 714 specimens were obtained from women and men, respectively. The most isolated microorganisms belonged to the blood samples (94.8%). *S.aureus* (32.6%) was the most prevalent isolates from all recovered samples. Microorganisms were detected in 1.2%, 1.6%, 1.61% and 2.9% of bone marrow, pleural effusion, vaginal and CSF samples, respectively (Table 1). 46.8%, 50.3% and 2.9% isolates belonged to gram-negative bacteria, gram-positive bacteria, and *Candida albicans*, respectively. Only one *C.albicans* isolate (11.1%) showed resistance to fluconazole, while the other 8 (88.89%) isolates were susceptible to this antibiotic. Among gram-negative bacteria, the frequency of *E.coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* were 20.9%, 19.5%, 17.4%, 14.6% and 11.9%, respectively. Also, *S.aureus* (64.9%), *S.epidermidis* (22%) and *Enterococcus* spp. (5.1%) were the most common isolated Gram-positive bacteria (Table 1). The results of the Antibiotic susceptibility tests are shown in Table 2. Among the Gram-positive bacteria, Diphtheroids and *Enterococcus* showed high resistance to rifampin, while *S.aureus* and *S.epidermidis* were highly resistant to ampicillin and amoxicillin (Table 2). The most resistant and sensitive gram-negative bacteria were *Acinetobacter baumannii*, and *salmonella* spp., respectively. Cephalexin and Ofloxacin also showed significant inhibitory effects (over 90%) on *E.coli* (Table 2).

4. Discussion

In the recent years, antibiotics have been important in the prevention and treatment of infectious diseases. Nevertheless, antibiotic resistant bacteria are considered as a serious problem in the world. Today, about seventy percent of the bacteria that cause infections in hospitals are resistant to at least one of the main drugs used for treatment (Julian and Dorothy, 2010). Therefore, it is important to have an overview and awareness of a new pattern of antibiotic resistance to improve health conditions and avoid extra expenses in prevention and treatment of infectious diseases.

In the present study, *S.aureus* was the highest (32.6%) and *Morganella morganii* and *Providencia rettgeri* were the lowest (0.3%) isolated strains. This findings are consistent with the results obtained by Streit et al. (Streit et al., 2004) and Sader et al. (Sader et al., 2005), while conflict with those reported by Sedighian et al (*E.coli* 33%) (Sedighian et al., 2008) and Jennifer et al (*E.coli* 21%) (Jennifer et al., 2004). In our study, 87.5% and 19% of *Acinetobacter baumannii* and *P.aeruginosa* isolates were resistant to imipenem, respectively. 24% and 100% of *E.coil* isolates were resistance to imipenem and cephalexin, while 3.6% and 56% of *S.aureus* isolates were resistant to ampicillin and clindamycin, respectively. In the study performed by Sader and his colleagues, 27-56% of *Acinetobacte baumannii* were resistant to imipenem, while up to 99% of *E.coli* were sensitive to this antibiotic (Sader et al., 2013). Haddadi et al. reported 79% resistance to imipenem in *Acinetobacter baumannii*, 10% in *E.coli*, 30% in *P.aeruginosa* and 7% in *Klebsiella pneumoniae* (Haddadi and Rasouli, 2007). In the study carried out by Tangden, the highest rates of resistance toampicillin, amikacin and ciprofloxacin were 85.3%, 6.6% and 10.2%, respectively (Tangden, 2014). In the present study, vancomycin was the most effective antibiotic against *Staphylococcus aureus* (95% sensitivity) that is consistent with those reported by with Sedighian (94% sensitivity) (Sedighian et al., 2008). Also, Mostafavi and his colleagues showed 22% resistance to vancomycin in *S.aureus* strains (Mostafavizadeh, 2007).

However, Alghaithy et al. reported no resistance to vancomycin in *S.aureus* strains. This conflict may be due to the Differences in health standards of geographic area (Alborzi et al., 2000; Alghaithy et al., 2000). Moreover, no VRSA detection in the study done by Alborzi can be because of the type of samples.

Antibiotic susceptibility pattern is variable around the world because of misuse, overuse and abuse of antibiotics, arbitrary administration, use of antibiotics as supplement in animal feeds, geological regions and level of hygiene.

Table 1. The number (%) organism isolated from the collected samples

CSF	Vaginal	Plural	Bone marrow	Blood	Type of sample
Number (%)	Number (%)	Number (%)	Number (%)	Number (%)	**Organisms**
1(0.3)	2(0.6)	0(0)	3(0.96)	94(3.03)	*S.aureus*
0(0)	0(0)	0(0)	1(30)	33(8)	*S.epidermidis*
0(0)	0(0)	0(0)	0(0)	8(2.5)	*Enterococcus*
0(0)	0(0)	0(0)	0(0)	4(1.3)	Diphtheroids
1(0.3)	0(0)	0(0)	0(0)	7(2.2)	Non-hemolytic *Streptococcus*
0(0)	0(0)	2(0.6)	0(0)	23(7.4)	*Pseudomonas*
0(0)	1(0.3)	0(0)	0(0)	28(9)	*E.coli*
0(0)	0(0)	0(0)	0(0)	28(9)	*Enterobacter*
0(0)	0(0)	1(0.6)	0(0)	16(5.2)	*Acinetobacter*
0(0)	1(0.3)	1(0.3)	0(0)	19(6.1)	*Klebsiella*
0(0)	0(0)	0(0)	0(0)	7(2.2)	*Brucella*
0(0)	0(0)	0(0)	0(0)	3(0.96)	*Salmonella*
0(0)	1(0.3)	0(0)	0(0)	2(0.6)	*Proteus*
0(0)	0(0)	0(0)	0(0)	4(1.3)	*Alcaligenes*
0(0)	0(0)	1(0.3)	0(0)	3(0.69)	*Citrobacter*
0(0)	0(0)	0(0)	0(0)	1(0.3)	*Morganella*
0(0)	0(0)	0(0)	0(0)	1(0.3)	*Providencia*
0(0)	0(0)	0(0)	0(0)	9(2.3)	*Candida albicans*
2(0.6)	5(1.6)	5(1.6)	4(1.03)	290(94.7)	The number and percent of total

Table 2. Percentage of antibiotic resistance

C.albicans	*M.morganii*	*P.retgeri*	*Salmonella spp.*	*K.pneumonia*	*A.baumannii*	*E.coli*	*P.aeruginosa*	*E.aerogenes*	*E. faecalis*	*S.epidermidis*	*S.aureus*	Antibiotic Organisms
-	100	0	-	-	-	0	-	-	100	43	26.6	Rifampin
-	-	-	-	50	100	40	0	100	50	39.1	25	Cephalothin
-	-	-	-	-	-	0	100	-	28.5	0	5.4	Vancomycin
-	-	100	-	-	-	-	-	-	57	98	90.8	Penicillin
-	-	-	0	-	-	-	-	-	50	68.2	62.3	Ampicillin
-	100	100	-	-	-	0	-	-	43	50.6	62.3	Amoxicillin
-	-	-	-	-	-	-	100	-	100	83.5	75.2	Erythromycin
-	100	-	0	22.2	91	69.5	0	0	86	54.6	56	Clindamycin
-	0	0	0	23.5	80	0	28.5	0	83	57.6	49.4	Ciprofloxacin
-	0	0	-	-	-	0	-	-	83	64	40.2	Cefoxitin
-	-	0	0	42.1	93	74	75	0	0	43	45	Cefazolin
-	0	0	0	60	100	100	100	0	-	0	50	Gentamicin
-	-	0	0	28.6	93.7	0	24	0	50	33.3	62.5	Tetracycline
-	-	0	0	19	78.5	24.1	19	0	-	33.3	56.5	Co-trimoxazole
-	0	-	0	50	100	89	35.7	0	-	83.5	61.5	Methicillin
-	-	-	0	43	94	75	56.2	33.3	50	33.3	83.3	Cephalexin
-	100	0	0	33.3	100	44.4	53.3	70	-	-	100	Imipenem
11.11	-	-	-	-	-	-	-	-	-	-	-	Fluconazole

Conclusions

Antibiotic resistant bacteria are increasing and considered as a serious problem in the world. Determining the antibiotic susceptibility pattern is necessary to avoid inappropriate use of antibiotics, prevent the resistant strains, and treat infectious diseases.

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Conflict of interest

All authors declare no conflict of interest.

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