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Original Article

Type of Bacterial Isolates and Antibiotic Resistance Patterns from Clinical Specimens in Yazd, Iran

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Background and Aims: Due to a wide variety of causes, various bacteria can acquire resistance to antibiotics. This study investigated the antibiotic susceptibility patterns of the etiologic bacterial agents of various infections in patients referred to three hospitals in Yazd city, Iran.

Materials and Methods: A total of 336 clinical specimens including wound discharge, sputum, blood, bronchial fluid, pleural fluid, ascitic fluid, synovial fluid, stool, and trachea secretions were collected in three hospitals. Microbiological culturing in order to grow and identify the causative bacteria were performed. Antibiotic susceptibility determinations were done by the Kirby Bauer disk diffusion method.

Result: Among all organisms isolated, *Escherchia coli*, *Staphylococcus. aureus*, *Staphylococcus. suprophyticus*, *Acinetobacter spp*., *Pseudomonas spp*., *Klebsiella spp*., *Staphylococcus. epidermidis* and *Entrococcus spp*. were the most prevalent, respectively. The *Escherchia coli* isolates were the most antibiotic-resistant Gram-negative bacterium. It seems that the same organisms recovered from different hospital wards show different antibiotic susceptibility patterns.

Conclusion: The increased prevalence of resistant organisms in different hospitals may reflect a lack of proper antibiotic usage policy, resulting in the prolonged and indiscriminate use of antimicrobial agents.

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Introduction

Microbiological culture is a primary method of letting organisms multiply in culture media under controlled *in vitro* conditions [1]. This technique is used to determine the type of organism in specimens isolated from patients such as blood, wound discharge, bronchial, pleural or ascitic fluid, etc., in order to determine the probable cause of infection [2].

Antimicrobial resistance is not new; however, the magnitude of resistant organisms, the geographic locations affected by drug resistance, and the breadth of resistance in single organisms are unprecedented and mounting [3]. Over time and due to a wide variety of causes, different bacteria can acquire resistance to various antibiotics [4]. An important task of clinical microbiology laboratory is the antimicrobial susceptibility testing of bacterial isolates from clinical specimens [5]. The tests will detect possible drug resistance in common pathogens and ensure susceptibility to drugs of choice for particular infections. They are performed to determine which antibiotic will most successfully treat a bacterial infection *in vivo* as susceptibility can vary even within a species [6, 7]. Diseases once thought to be controlled by particular antibiotics are becoming increasingly resistant to standard therapies. Drug-resistant strains initially appeared in hospitals where most antibiotics were being used [8]. Resistance to multiple drugs was first detected among enteric bacteria, namely, *E. coli*, *Shigella spp*., and *Salmonella spp*. in the late 1950s to early 1960 [9, 10]. The use of broad-spectrum antibiotics has been the most important cause of antibiotic resistance in recent years [11]. The emergence of resistant bacteria and the problems these persistent bacteria cause in treating patients urge the necessity for precise knowledge about these bacteria and their susceptibility patterns [4, 11, 12]. Attention to microbial antibiotic responses can help control disease outbreak in hospital settings [13].

Administration of effective antibiotics not only can improve the quality of patient treatment but also can reduce medical costs. This study aimed to determine the antibiotic susceptibility patterns of bacterial isolates from patients in three different hospitals in Yazd city, Iran.

Materials and Methods

Study Area

Clean catch clinical specimens were obtained from 336 patients (223 men and 113 women) referred to different wards of three hospitals in Yazd city, Iran. The study was conducted during ten months period (from March 2015 to January 2016). All experiments were performed in the department of microbiology laboratory, faculty of medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Sample collection / Study design

Patient specimens which included wound discharge, sputum, blood, bronchial fluid, pleural fluid, ascitic fluid, synovial fluid, pleural fluid, stool, and trachea secretions were collected from 336 hospitalized patients. Selections were made irrespective of the

infection's cause, and patient age ranged from 6 to 87 years. The exclusion criteria were the lack of antibiotic therapy prior to sample collection. Each sample was collected in a 20 ml calibrated sterile container. The specimens were appropriately labeled, transported to the laboratory immediately, and analyzed within 3 hours after collection.

Sample processing/culture and identification of organisms

Culture and antibiotic susceptibility tests were performed on clinical specimens obtained from the patients before initiation of any antibiotic therapy. Each sample was aseptically inoculated into blood agar and MacConkey agar differential media on arrival at the laboratory. The plates were incubated aerobically at 37 °C for 24-48 hours. Then, the characteristic bacterial isolates observed on the differential media plates were sub-cultured onto new culture media plates. Any grown bacteria were subjected to microscopical and appropriate selective tests such as catalase and coagulase for Gram-positive and IMVIC for gram-negative bacteria to identify each organism correctly.

Antibiotic susceptibility test

Bacterial identification was made based on cultural and biochemical characteristics [14]. The following antibiotic disks were used for each bacterium based on their Gram stain and clinical sources: Tetracycline (30µg), Ceftriaxone (30µg), Ciprofloxacin (5µg), Ceftizoxime (30µg), Amikacin (30µg), Chloramphenicol (30µg), Cephalothin (30µg), Nalidixic acid (30µg), Ceftazidime (30µg), Amoxicillin-clavlanate (30µg), Imipenem (10µg), Ampicillin (10µg), Norfloxacin (10µg), Nitrofurantoin (300µg), Carbenicillin (100µg), Gentamicin (10µg), Meropenem (10µg), Clarithromycin (15µg), Clindamycin (2µg), Azithromycin (15µg), Vancomycin (30µg), Oxacillin (1µg), Penicillin (10µg), Polymyxin B (300µg), Piperacillin (100µg), Doxycycline (30µg), Ticarcillin (75µg), Cefotaxime (30µg), Cefazolin (30µg), Cefuroxime (30µg), Cefepime (30µg), Cefixime (5µg), Ofloxacin (5µg), Tobramycin (10µg), Streptomycin (10µg), Cefoxitin (30µg), Colistin (10µg), Rifampin (5µg), Trimethoprim-Sulfamethoxazole (1.25/23.75µg), Levofloxacin (5µg), Fosfomycin (200µg), Ampicillin-Sulbactam (10/10µg), Aztreonam (30µg), Trimethoprime (5µg), and Erythromycin (15µg). In the disk diffusion assay used in this study, within 15 min after applying the antibiotic discs (Oxoid Co., Australia), the plates were incubated at 37 °C. After 24h of incubation, plates were examined, and the diameter of growth inhibition zones was measured. The zone diameter for individual antimicrobial agents was then translated into susceptible, intermediate, and resistant categories based on Clinical and Laboratory Standards Institute 2015 criteria.

Institutional Review Board approved this study, so there was no deviation from the prescribed standard protocol of treatment, and it was conducted after obtaining due ethical approval from hospital administration.

Results

The present study includes culture information and antimicrobial susceptibility data for each bacterial isolate obtained from patients hospitalized for 72 hrs or more in hospitals of

Yazd. The age of patients in this study ranged from newborn to 87 years (Figure 1).

The distribution of patients from whom specimens were taken in each hospital ward is shown in Table 1. From the 336 positive urine cultures, 22 different bacterial isolates were obtained. *E. coli* was the predominant and most frequently isolated pathogen, followed by *S. aureus*. Figure 2 shows the details of cultured bacteria. Among all the organisms isolated from patients, the eight most frequent organisms were chosen and subjected to an antibiotic susceptibility test. Bacterial susceptibility to tetracycline and Ceftriaxone was summarized in Table 2, and susceptibility to ampicillin and gentamicin was shown in Table 3.

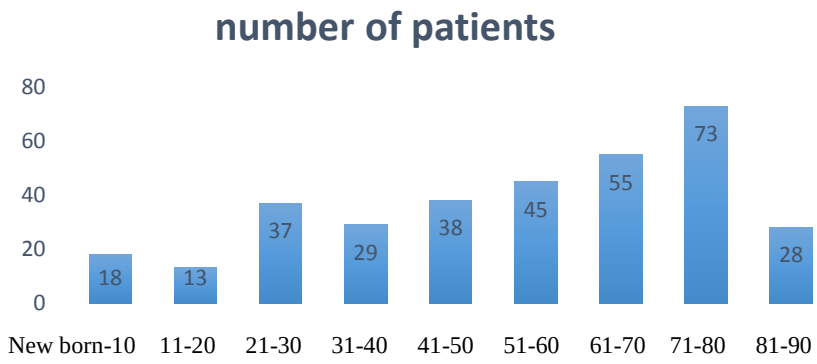

Fig. 1. The age group of patients

Table 1. Distribution and number of patients in different hospital wards

Part of hospital	Number of patients
Intensive care unit	73
Internal	49
Surgery	66
Cardiac care unit	4
Very important person	7
Gynecology	2
New born	6
Pediatrics	2
Neonatal intensive care unit	4
Infectious	86
Urology	14
Surgical intensive care unit	10
Central lab	13
Total	336

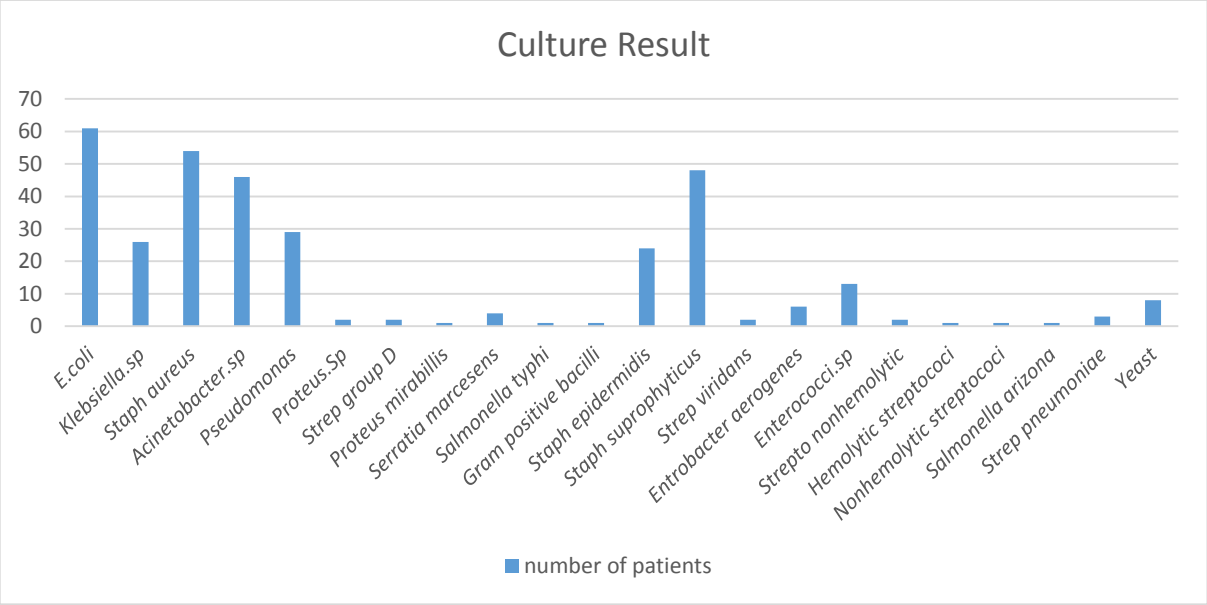

Fig. 2. Organisms isolated in the study population

Table 2. Susceptibility to Tetracycline and Ceftriaxone

	Tetracyclin			Ceftriaxone		
Pathogen	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	Resistant
E.coli	2	33	25	9	21	30
Staph aureus	3	18	33	24	9	21
***Acinetobacter*.sp**	2	19	24	3	5	37
Pseudomonas	20	0	8	10	6	12
Staph epidermidis	7	11	6	3	17	4
Staph suprophyticus	8	25	14	12	19	16
***Klebsiella*.sp**	6	5	15	5	8	13
***Enterococci*.sp**	5	4	4	1	7	5

Table 3. Susceptibility to Ampicillin and Gentamicin

	Ampicilin			Gentamicin		
Pathogen	Intermediate	Sensitive	Resistant	Intermediate	Sensitive	Resistant
E.coli	9	10	41	12	28	20
Staph aureus	6	14	37	16	33	8
***Acinetobacter*.sp**	5	3	37	3	3	39
Pseudomonas	5	3	21	5	20	4
Staph epidermidis	4	7	13	0	19	5
Staph suprophyticus	8	14	25	9	21	17
***Klebsiella*.sp**	6	2	18	1	17	8
***Enterococci*.sp**	3	6	4	0	8	4

Discussion

Infectious diseases by resistant bacteria have become an extraordinary predicament in all medical and therapeutic centers [15]. The appearance of antibiotic-resistant bacterial strains has become a worldwide danger [16, 17]. Development of antibiotic resistance in particular stems from drugs targeting only specific bacterial molecules [18]. Because the drug is so specific, any mutation in these molecules will interfere with or negate its destructive effect, resulting in antibiotic resistance [19, 20]. Many investigators have

demonstrated that *E. coli*, *S. aureus*, *Pseudomonas* spp., and *Klebsiella* spp. are frequently isolated from hospitalized patients [21-24]. In the present study, eight organisms were more common among the 23 organisms isolated from patients. Sixty patients from 336 showed evidence of urinary tract infections (UTI) due to *E. coli* (17.8%). *S. aureus* was the next prevalent isolate (16.9%), followed by *S. saprophyticus* (13.9%), *Acinetobacter* spp. (13.3%), *Pseudomonas* spp. (8.6%), *Klebsiella* spp. (7.7%), *S. epidermidis* (7.1%) and *Enterococcus* spp. (3.8%), respectively. Most patients in our study were between 71 to 80 years of age (21.7%). Furthermore, they were mostly hospitalized in infectious diseases wards (25.5%).

When whole populations are treated with the same class of antibiotics, susceptible strains will have little opportunity to recolonize their niche, and resistant strains will acquire an important advantage [25, 26]. *E. coli* is an increasingly prevalent antibiotic-resistant Gram-negative bacterium in hospitals. This bacterium causes UTI, gastroenteritis, septicemia, and meningitis. The *E. coli* isolates in this study were uniformly resistant to beta-lactam antibiotics such as ampicillin (68% of all isolated *E. coli*) and Ceftriaxone (50% of all isolated *E. coli*; however, they were somewhat sensitive to tetracycline and gentamicin. The *S. aureus* isolates were sensitive to gentamicin (70.2% of all isolated *S. aureus*), very resistant to tetracycline (61.1%), and have shown outstanding intermediate resistance to Ceftriaxone (44.4%). Acinetobacter isolates were resistant to almost all the antibacterial agents. *S. saprophyticus* isolates were sensitive to tetracycline (53.2%), ceftriaxone (40.4%), and gentamicin (44.6%) but resistant to ampicillin (53.1%). Pseudomonas isolates were sensitive to gentamicin (68.9%) while resistant to ampicillin, ceftriaxone, and tetracycline. The same organism in different wards of hospitals shows different antibiotic resistance patterns. This may be due to three main reasons: first, drug inactivation or modification (e.g., enzymatic deactivation of penicillin G in some penicillin-resistant bacteria through the production of β-lactamases). Second, alteration of the target site (e.g., alteration of the binding target site for penicillin). Third, alteration of metabolic pathways [26-28].

Conclusion

The increased prevalence of resistant organisms in different hospitals may reflect a lack of proper antibiotic usage policy, resulting in the prolonged and indiscriminate use of antimicrobial agents.

Conflict of interest

The authors declare have no conflict of interest to declare.

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