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Original Article

# Surveillance of current antibiotic resistance among clinical isolates S. aureus, E. coli and P. aeroginosa collected from five Iranian cities

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## Abstract

Antibiotic resistance is an emerging problem worldwide present in many bacteria, specially S. aureus, P. aeroginosa, and E. coli that are consider as the most common group of bacteria responsible for nosocomial infections. This problem would be more serious and divesting in developing countries where there is not regular surveillance program for periodic antibiotic resistance. In attempts to report the antibiotic pattern in Iranian cities, we collected clinical isolates and tested them against current antibiotics. 653 Clinical isolates of S. aureus, P. aeroginosa, and E. coli were collected during 5 months. The isolates were then transported to research laboratory within glycerol containing medium. Standard antibiotic discs containing different concentrations of each agent were provided from MAST media. Standard disc diffusion method was carried out and the result was interpreted using NCCLS tables and data charts. Isolates were mostly cultured from respiratory, Urine, wounds, blood clinical samples. The clinical isolates were E. coli (45%) S. aureus (33%), and P. aeroginosa (22%). 20% of E. coli, 9% of P. aeroginosa, and 12% of S. aureus isolates showed reduction of sensitivity to Amikacin (AK). 27% of S. aureus had reduction of sensitivity to Vancomycin (VA). About 25% of P. aeroginosa showed reduced sensitivity to Gentamicin (GM).

**Key words:** Antibiotic resistance, Amikacin, Vancomycin, Gentamicin, S. aureus, P. aeroginosa, E. coli, Iran

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## 1. Introduction

Antibiotic resistance is an emerging problem worldwide present in many bacteria. Nosocomial infections occurs worldwide and affect both developed and resource poor countries. The most common group of bacteria responsible for nosocomial infections are Staphylococcus aureus, Pseudomonas aeroginosa, and Escherichia coli. The emergence of resistant strains has increased the morbidity and mortality associated with infections of different human organs especially; urinary tracts, trauma, burns, wounds, respiratory tracts, sepsis (Syeda et al. 210, Goswami et al. 2011).

P. aeruginosa is an epitome of opportunistic nosocomial pathogen, which causes a wide spectrum of infections and leads to substantial morbidity in immuno compromised patients (Lee et al. 2009). Despite therapy the mortality due to Nosocomial, Pseudomonal pneumonia is approximately 70%.(Castre et al. 2000). Unfortunately, P. aeruginosa developed resistance to most of antibiotics thereby jeopardizing the selection of appropriate treatment (Obritsch et al. 2004). The S. aureus strains are beginning to develop resistance to vancomycin in different regions of the world. the emergence of VRSA/VISA (Vancomycin resistant Staphylococcus aureus/vancomycin intermediate Styphylococcus aureus) might also be prevalent as antibiotic misuse is equally common (Tiwari et al. 2006). E. coli has high percentages of resistance to ampicilline, thrimethoprim-sulfamethoxazole, tetracycline, chloramphenicol and nalidixic acid, which implies important complications in antibiotic treatment when required (Giacometti et al. 2000). Escherichia coli represents an incredible versatile and diverse enterobacterial species and can be subdivided into the following; (i) intestinal non-pathogenic, commensal isolates. (ii) Intestinal pathogenic isolates and (iii) extraintestinal pathogenic E. coli or ExPEC isolates (Goswami 2011). They are three most frequent nosocomial infection, associated with increased hospital stay, costs, and use of antimicrobial agents (Mangram et al. 1999). Antibiotic resistance can be controlled by appropriate antimicrobial prescribing, prudent infection control, new treatment alternatives, and continued surveillance (Ducel et al. 2001). The increasing antibiotic resistance is due to the acquisition of different molecular mechanisms of resistance through point chromosomal mutations and / or horizontal transfer of genetic material between related or different species facilitated by some genetic elements such as integrons (Weber et al. 2009). Risk factors other than microbiology can be due to systemic factors affecting the patient's healing response, local wound characteristics, or operative characteristics. Its risk depends on bleeding, the amount of devitalized tissue created, the need for drains within the wound, obesity and diabetes mellitus (Suchitra et al. 2009). Due to significant changes in microbial genetic ecology, as a result of indiscriminate use of anti-microbials, the spread of antimicrobial resistance is now a global problem (Schmitz et al. 1999). Present study was preliminary designed to find out the antibiotic susceptibility patterns of P. aeroginosa, S. aureus, and E. coli clinical isolates collected from different major cities in Iran.

## 2. Materials and methods

Different strains of P. aeroginosa, S. aureus, and E. coli were isolated from clinical samples collected within each city's medical laboratory during last periods of 2011. The isolated were characterized and microbiologically identified at the species level in original laboratory prior shipment to central research laboratory in Tehran. All strains were transported to destination within in glycerol containing media and through standard circumstances.

The isolates were then recovered on standard laboratory media (Sheep Blood Agar and EMB agar) for each species and reconfirmed their preliminary identification. The tablets and standard discs containing different concentrations of Amikacin (AK), Ampicilin (AMP), Cefotaxime (CTX), Cefpirome (CEP), Ceftazidime (CAZ), Ceftrixone (CRO), Cephazolin (CZ), Cloramphenicol (CL), Ciprofloxacin (CIP), Clindamycin (CD), Cloxacillin (CX), Gentamicin (GM), Imipenem (IM), Tazobactam /Piperacillin (PTZ), Vancomycin (VA) were purchased from MAST Media (MAST, UK. LTD), and were kept according the manufacturer instruction until use. Standard disc diffusion method (Kerbi-Byer disc diffusion method) was carried out and the result was interpreted using National Committee for Clinical Laboratory Standards (NCCLS) tables and data charts (Wkler et al. 2005). The resistance isolates were then subjected to MIC tests, according to supplier's manual.

## 3. Results

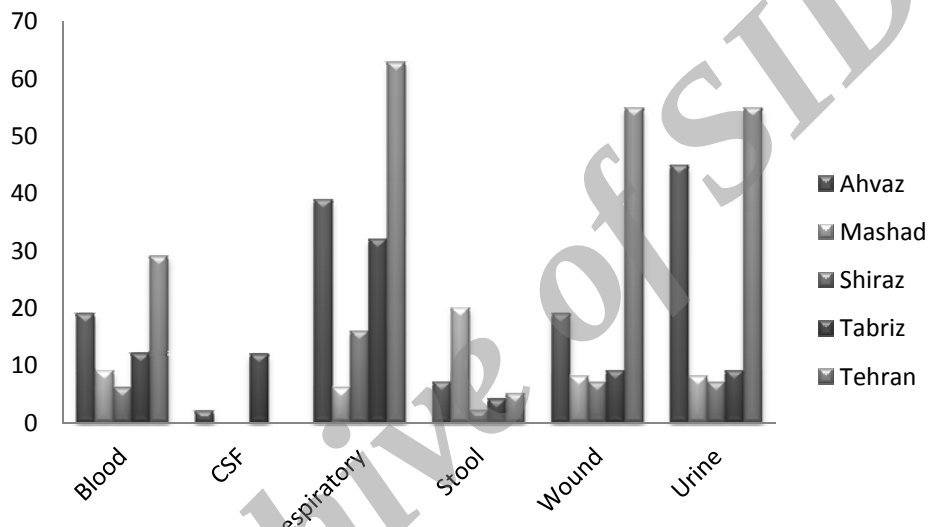


Figure 1: Comparison clinical sources of isolates

The isolates were obtained from different Clinical samples

Isolates were mostly cultured from respiratory, Urine, wounds, blood, CSF, stool by; 34%, 22%, 21%, 15%, 3.7%, and 3.4% respectively. Totally 653 isolates were subjected to experimental analysis in our study, which

Table 1: Distribution of isolates through origin cities of collection

| spp | Ahvaz | Mashad | Shiraz | Tabriz | Tehran | Total |
|---|---|---|---|---|---|---|
| P. aeroginosa | 76 | 41 | 24 | 22 | 63 | 216 |
| S. aureus | 16 | 72 | 25 | 16 | 12 | 143 |
| E. coli | 108 | 36 | 26 | 27 | 96 | 294 |
| Total | 200 | 148 | 75 | 67 | 163 | 653 |

were contributed almost equally by five cities located in north, center and south of nationwide

The clinical isolates were commonly identified as E. coli (45%) S. aureus (33%), and P. aeroginosa (22%).

More than 60% of E. coli were sensitive to Amikacin (AK) and 20% were resistant to it, 20% of isolates showed reduction of sensitivity. 50% of E. coli were sensitive to Ampicilin (AMP), and 20% were resistant to (AMP), remaining isolates showed reduction of sensitivity. 54% of E. coli were sensitive and 46% were resistant to Cefotaxime (CTX). 62% were sensitive to Ciprofloxacin (CIP), the of remaining isolates showed reduction of sensitivity. 70% were sensitive to Cloramphenicol (CL) and 30% were resistant to it, remaining isolates showed reduced of sensitivity. (GM), 55% of E. coli were sensitive to Gentamicin (GM) and 25% were resistant to it, remaining isolates showed

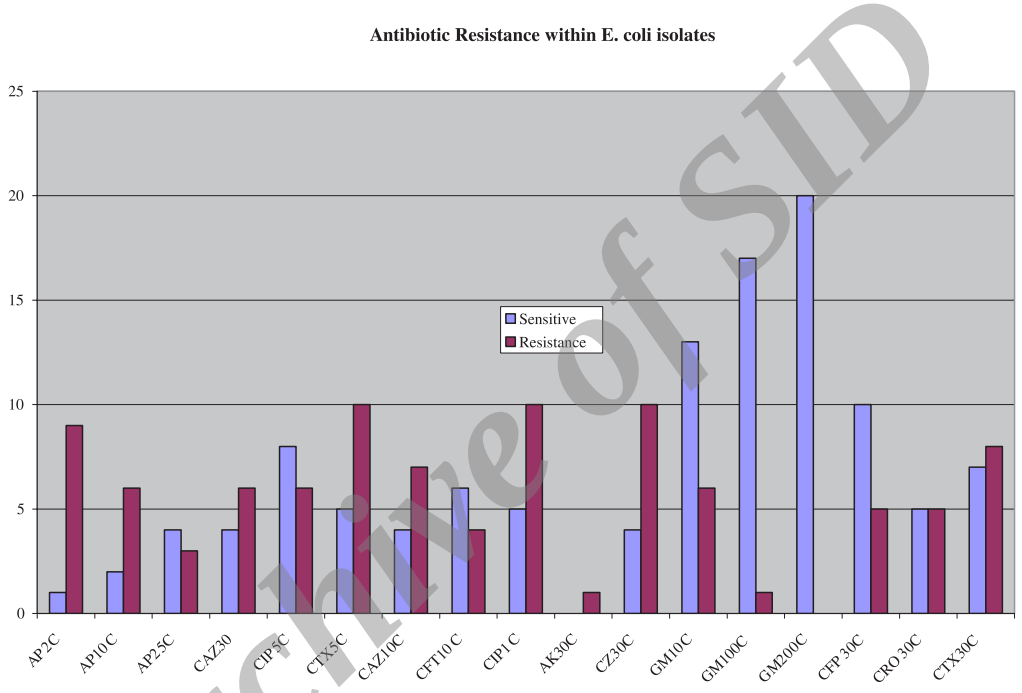


Figure 2: Pattern of antibiotic resistance in E.coli isolates

reduction of sensitivity. 81% of E. coli were resistant to Imipenem (IM), the remaining isolates showed reduction of sensitivity

About 54% of P. aeroginosa were sensitive to, and 37% were resistant to Amikacin (AK), 9% of isolates showed reduction of sensitivity. 40% were sensitive, and 40% were resistant to Ampicilin (AMP), the remaining isolates showed reduction of sensitivity. P. aeroginosa (54%) were sensitive and 46% were resistant Cefotaxime (CTX). 33% were sensitive and 61% were resistant Ceftazidime (CAZ), the remaining 6% showed reduction of sensitivity. (CIP), 24% of P. aeroginosa were sensitive to Ciprofloxacin (CIP), 39% were resistance, and the remaining isolates showed reduction of sensitivity. Cloramphenicol (CL) sensitivity was about 70% within P. aeroginosa isolates, while 30% were resistant, and the remaining isolates showed reduction of sensitivity. 75% of were sensitive to Gentamicin (GM), and remaining isolates were resistance. Imipenem (IM) sensitivity was at least 68% among P. aeroginosa isolates, and remaining were resistance or reduced the sensitivity to IM. Tazobactam /Piperacillin (PTZ) affected

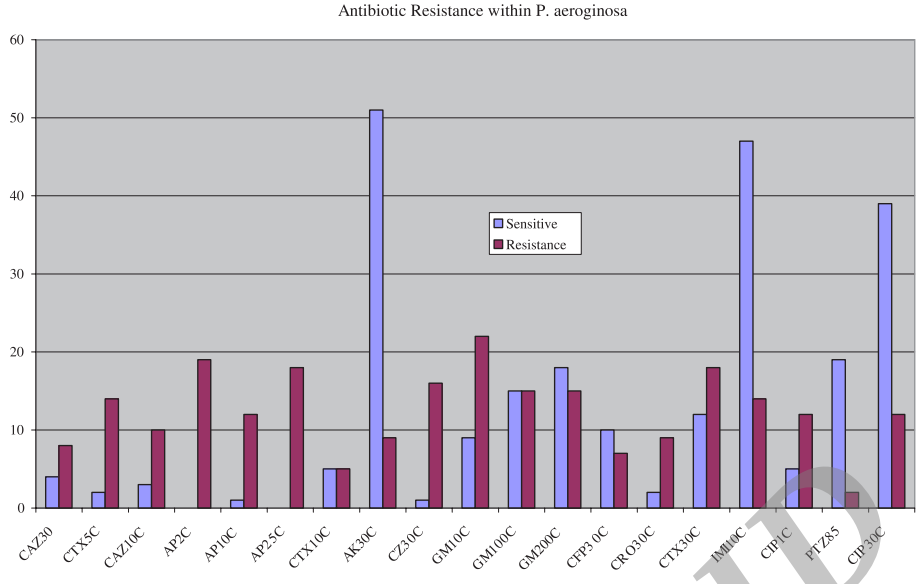


**Figure 3: Pattern of antibiotic resistance in P. aeroginosa isolates**

successfully on 37% of isolates, while 44% were resistant to it, and the remaining isolates showed some degree of sensitivity reduction

More than 58% of S. aureus were sensitive to Amikacin ( AK) and 20% were resistant to it, and 12% of isolates showed reduction of sensitivity. 50% of isolates were sensitive and 20% were resistant Ampicilin (AMP), the remaining isolates showed reduction of sensitivity. Cefotaxime (CTX), 54% of S. aureus were sensitive to CTX and 46% were resistant to it. Ciprofloxacin (CIP); 62% of S. aureus were sensitive to CIP the remaining isolates showed reduction of sensitivity. Clindamycin (CD); 63% of S. aureus were sensitive to CL and 30% were resistant to it, remaining isolates showed reduction of sensitivity. Cloramphenicol (CL); 70% of S. aureus were sensitive to CL and 30% were resistant to it. Cloxacillin (CX); 16% of isolates were sensitive to CL and 84% were resistant to it. Gentamicin (GM); 45% of S. aureus were sensitive to GM and 30% were resistant, remaining isolates showed reduction of sensitivity. Imipenem (IM); 81% of S. aureus were resistant to IM, and the remaining isolates showed reduction of sensitivity. About

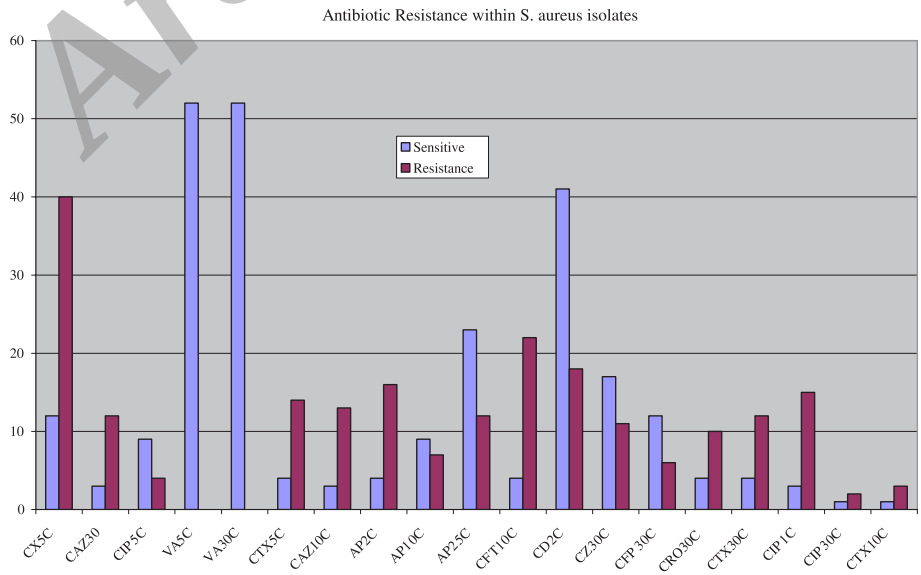


**Figure 4: Pattern of antibiotic resistance in S. aureus isolates**

60% of isolates were sensitive and 13% were resistant to Vancomycin (VA), remaining isolates showed reduction of sensitivity

## 4. Discussion

The aim of present study was to survey sensitivity pattern of clinical isolates of three major causes of nosocomial infections. Bacterial isolates were mostly cultured from clinical samples such as respiratory secretions, urinary tracts, wounds, and blood. Although the wide spectrum of gram positive and gram negative bacteria were isolated from clinical samples, and interesting information produced (data not shown), but our logic emphasize was on E. coli, P. aeroginosa, and S. aureus. E. coli was the most organism isolated followed by P. aeruginosa, an d S. aureus; while the predominant isolates was subject to changes from sample to sample. Similar findings were observed in previous studies where percentage of gram negative bacilli from the wounds was more, and coagulase positive Staphylococcus was predominant organism (Rubin et al. 2006). We observed reduction of sensitivity within Staphylococcus aureus to Amikacin (12%), Ampicilin (30%), Ciprofloxacin (38%), Clindamycin (7%), Gentamicin (25%), Imipenem (19%), and Vancomycin (27%). It is essential for clinical laboratories to screen for and confirm vancomycin resistance in the clinical laboratory

Incidence of vancomycin resistance S. aureus (VRSA) in our study was slightly higher than previously reported data of India.(Tiwari and Sen 2006). This should be cautiously interpreted as this might be because of false positive result by other species (Tiwari and Sen 2006) . Other study also shows false positive result by using same disc diffusion method (Hiramatsu et al. 1977) studied that Styphylococcus aureus was also capable of transformation into the homogenously resistant strains and adhering to artificial surfaces, The BSAC (British Society for Antimicrobial Chemotherapy) has standardized method for disc diffusion testing to evaluate vancomycin susceptibility for MRSA isolates.(Howe et al. 1999). There is a need for the modern day clinical laboratory to develop a definitive method to confirm hetero-resistance to vancomycin among MRSA isolates as this could direct antibiotic therapy. However, there is need for active VRSA surveillance by healthcare institutions in India to enforce infection control measures, rational use of antibiotics and to prevent development of further resistance against this organism (Lyer et al. 2008).

In the present study, Pseudomonas aeruginosa showed reduced sensitivity to commonly used antibiotics such as Amikacin, Ampicilin, Cephotaxime, and ciprofloxacim. It is more and less, in agreement to previously published reports (Lee et al. 2011, Burnham et al. 2010). Ciprofloxacin has been stated to be the most potent drug available for the treatment of P. aeruginosa infections (Chastre et al. 2000 , Obritsch et al 2004). This report is in conformity with the result of our study in which ciprofloxacin resistance was 39% among P. aeruginosa isolates from wound infection patients. Similar reduced resistance of P. aeruginosa to ciprofloxacin has been reported in Jamaica, Latin America , Nigeria and in Malaysia ( Abu hanifa 1990, 24). It is undoubtable that at the present time, ciprofloxacin is the most effective antibiotics against P. aeruginosa involved in wound infection relative to most other commonly used drugs (Zhanel et al. 2008). Periodic susceptibility testing should be carried out over a period of two to three years to detect the resistance trends (Andrews 2001). Also, a rational strategy on the limited and prudent use of antipseudomonal agents is urgently required. In fact, the irrational and inappropriate use of antibiotics is responsible for the development of resistance of Pseudomonas species to antibiotic monotherapy. Pseudomonas resistant to carbapenem and third generation cephalosporins is real threat (Gales et al. 2001).

The incidence of P. aeruginosa, S. aureus and E. coli in Nosocomial and postoperative

wound infection is becoming more serious in developing countries because of relaxation in general hygienic measures, production of low quality antiseptic, and medicinal solutions for treatment, difficulties in proper definition of the responsibility among the hospital staff (Fadeyi and Akanbi 2005). Lack of uniform antibiotic policy and indiscriminate use of antibiotics may have lead to emergence of resistant bacterial strains. Particularly pseudomonas resistance to third generation cephalosporin and meropenem and VRSA/VISA are real threat to control hospital acquired infection (Ducel et al 2001). Hence, there should be an immediate response from the concerned authorities to check further emergence and spreading of these notorious VRSA strains and also there is a need to emphasize the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials (Schmitz et al. 1999). Here is an alarming increase of infections caused by antibiotic-resistant bacteria. Further studies are required to check the results provided in this study before final conclusion. In addition, regular antimicrobial susceptibility surveillance is essential for area-wise monitoring of the resistance patterns Sensitivity pattern of E. coli in our study confirmed the work of Syeda et al. as the sensitivity of Ciprofloxacin was 62% vs. 62.7%, and for Ampicillin was 50 vs. 51.2 (Syeda et al. 2010), but they were considerably less than information reported by Goswami et al. as 62 vs. 87-100 for Ciprofloxacin, and 50 vs. 83-84 for Ampicillin (Goswami et al. 2011). Interestingly, strains isolated from wounds infections were sensitive to CIP, CX, GM, and IM. An effective national and state level antibiotic policy and draft guidelines should be introduced to preserve the effectiveness of antibiotics and for better patient management.

## 5. Acknowledgment

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**Conflict of interests :** None declared.

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