ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 03, pp.13699-13702, March, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ANTIBIOTIC RESISTANCE PROFILE OF GRAM NEGATIVE BACTERIAL ISOLATES COLLECTED FROM VARIOUS HOSPITAL AND LABS OF KARACHI, PAKISTAN

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ARTICLE INFO

Article History:
Received 28th December, 2014
Received in revised form
27th January, 2015
Accepted 23rd February, 2015
Published online 31st March, 2015

Key words:

Gram negative bacteria,
Antibiotic, Resistance.

ABSTRACT

A total of 51 gram negative bacterial cultures including *E.coli, Pseudomonas, Salmonella, Enterobacter* and *Klebsiella* species were obtained from different labs and hospitals. The bacterial isolates were screened for their antibiotic resistance against six commonly used antibiotics i.e. Streptomycin, Sulzone, Cefotaxime, Fosfomycin, Amikacin, Tazocin at six different concentrations (3µg, 5µg, 10µg, 30µg, 50µg and 100µg/ml).The clinical isolates showed significant resistance to commonly used antibiotics and some of them were found to be multidrug resistant. The frequency of resistance of bacterial isolates to individual antibiotics is found to be 78% for Cefotaxime, 70% for Sulzone, 73% for Streptomycin, 52% for Fosfomycin, 48% for Tazocin and 23% for Amikacin. The resistance frequency of gram negative bacteria at a concentration of 100µg/ml of individual antibiotic was found to be 51% for Cefotaxime, 20% for Sulzone, 25% for Fosfomycin, 37% for Streptomycin, 4% for Tazocin and no resistance was noted in case of Amikacin.

INTRODUCTION

Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major healthcare problem in the 21st century. Unfortunately the antibiotic resistance is a widespread problem around the world affecting many people especially those who belong to developing nations (Alanis, 2005). The drug resistance observed in antibiotic era is related to the use of antibiotics in clinical and veterinary practices, animal husbandry and agriculture and it is supported by studies that have clearly correlated the emergence and dissemination of resistance with the use of antibiotics (Pallecchi *et al.*, 2007). Researchers seem that antibiotic resistance has emerged due to the disparity created in the microbial ecosystem caused by changes in the selective pressures applied by antibiotics on microbes (Gaur *et al.,* 2006). Bacterial species from clinical and non-clinical settings are becoming increasingly resistant to conventional antibiotics. 10 years ago, concern centered on Gram-positive bacteria, particularly methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*, but now clinical microbiologists increasingly agree that multidrug-resistant Gram-negative bacteria pose the greatest risk to public health. Not only is the increase in resistance of Gram-negative bacteria more rapidly than in Gram-positive bacteria (Cornaglia, 2009) but also there are fewer new and developmental antibiotics active against Gram-negative bacteria (Baiden *et al.,* 2010, Wise *et al.,* 2010) and drug development programs appear insufficient to endow with therapeutic cover in 10–20 years (Boucher, 2009, Rice 2009). There are three main types of antibiotic resistance namely, natural resistance, acquired resistance and plasmid mediated resistance (Arnold, 1980). In Pakistan multidrug resistant isolates of *E.coli* are much higher to 3rd generation cephalosporin and quinolones, during 1980-1990 there were reports of multidrug resistant *S.typhi* bearing R plasmid caused epidemic typhoid fever in Pakistan and India (Khan *et al.,* 1980; Pickering, 2004). During recent times new mechanism of resistance has given rise to the development of resistance to several classes of antibiotics creating multidrug resistant bacterial strains (Arnold 1980). The purpose of current study is to find out the antibiotic resistance profile of clinical gram negative bacteria against six commonly used antibiotics.

MATERIALS AND METHODS

Standard Bacterial Strains

The standard bacterial strains of *E.coli* (40MD and X103B) were included in the study. These strains came as a gift from Dr. Mary Berlyn of *E.coli* genetic resource centre, yale university USA.

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Collection of Clinical Gram negative bacteria

The Gram negative bacterial strains were obtained from SIUT hospital and Dr. Essa's Lab, Karachi. All the bacterial strains were collected on tryptone agar slants and purified twice on MacConkey's agar. Colonial characteristics and biochemical reactions of each isolate were noted.

Antibiotics

All the antibiotics including Streptomycin, Sulzone, Cefotaxime, Fosfomycin, Amikacin, Tazocin were purchased from local market. Antibiotic stock solution (10mg/ml) was prepared in sterile distilled water, sterilized by millipore filtration and kept frozen when not in use.

Determination of antibiotic resistance of bacterial strains

Antibiotic resistance test was performed by the method of replica plating using MacConkey's agar. A master plate was prepared on which the isolates were spotted. Standard bacterial strains were also plated as controls. After 24 hours of incubation at 37°C, impression of master plate was transferred onto MacConkey's agar plates containing different concentrations (3, 5, 10, 30, 50 & 100 µg/ml) of individual antibiotics. Control plates without antibiotic were also made with each replication. Results were recorded after 24 hours of incubation at 37° C.

RESULTS

Collection, Purification and Characterization of bacterial isolates from clinical sources

Total 51 gram negative bacteria were obtained from different hospitals and labs. On the basis of various biochemical tests, these were identified as *E. coli* (total 19), *Klebsiella* (total 7), *Enterobacter* (total 6), *Pseudomonas* (total 4) and *Salmonella* (total 1), while 14 remained unidentified.

Determination of Resistance Pattern

All the gram negative bacteria obtained from clinical sources were screened for their antibiotic resistance against six commonly used antibiotics namely Amikacin, Cefotaxime, Fosfomycin, Streptomycin, Sulzone and Tazocin. The resistances were tested at six different concentrations i.e; 3ug/ml, 5ug/ml, 10ug/ml, 30ug/ml, 50ug/ml and 100ug/ml. Of the total 51 bacteria collected, all were found resistant to one or more antibiotics (Table 1).

Resistance to 10ug/ml and more is considered significant. Frequency of resistance of bacterial isolates to individual antibiotics were found to be 78% for Cefotaxime, 73% for Streptomycin, 70% for Sulzone, 52% for Fosfomycin, 48% for Tazocine and 23% for Amikacin. The resistance frequency of gram negative bacteria at a concentration of 30ug/ml, 50ug/ml and 100ug/ml of individual antibiotic are presented in figure 1, 2 and 3 respectively.

Table 1. Resistance Pattern of clinical Gram negative bacteria*

S.No	Isolate No.	Resistance Pattern**
1	SM-1	$Am_3Cf_{100}Fm_{50}Sm_{10}Sz_{50}Tz_{50}$
2	SM-2	$Am_{10}Cf_{100}Fm_{50}Sm_{30}Sz_{50}Tz_{30}$
3	SM-3	$Am_5Cf_5Fm_3Sm_{100}Sz_3Tz_5$
4	SM-4	$Am_3Cf_{50}Fm_{50}Sm_{10}Sz_{10}Tz_5$
5	SM-5	$Am_3Cf_{10}Fm_{100}Sm_{10}Sz_{50}Tz_{10}$
6	SM-6	$Am_3Cf_{10}Fm_{100}Sm_{10}Sz_{50}Tz_{30}$
7	SM-7	$Am_3Cf_5Fm_{50}Sm_{100}Sz_{50}Tz_{50}$
8	SM-8	$Am_{30}Cf_{100}Fm_5Sm_{100}Sz_{30}Tz_{30}$
9	SM-9	$Am_5Cf_3Fm_3Sm_{100}Sz_{10}Tz_5$
10	SM-10	$Am_5Cf_{100}Fm_{100}Sm_{100}Sz_{50}Tz_{30}$
11	SM-11	$Am_5Cf_{30}Fm_{100}Sm_{10}Sz_{30}Tz_{50}$
12	SM-12	$Am_3Cf_{30}Fm_{100}Sm_{10}Sz_{100}Tz_{50}$
13	SM-13	$Am_5Cf_3Fm_3Sm_{10}Sz_{10}Tz_{50}$
14	SM-14	$Am_3Cf_5Fm_3Sm_{10}Sz_{10}Tz_{50}$
15	SM-15	$Am_3Cf_{50}Fm_5Sm_{50}Sz_{100}Tz_{50}$
16	SM-16	$Am_3Cf_{100}Fm_{30}Sm_{100}Sz_{30}Tz_3$
17	SM-17	$Am_3Cf_{100}Fm_{10}Sm_5Sz_{50}Tz_{10}$
18	SM-18	$Am_3Cf_{100}Fm_{50}Sm_{100}Sz_{100}Tz_3$
19	SM-19	$Am_3Cf_5Fm_3Sm_{10}Sz_3Tz_{50}$
20	SM-20	$Am3Cf_{30}Fm_{100}Sm_{10}Sz_{100}Tz_{10}$
21	SM-21	$Am_3Cf_{50}Fm_{100}Sm_{50}Sz_{100}Tz_{10}$
22	SM-22	$Am_{30}Cf_{100}Fm_5Sm_{100}Sz_{100}Tz_{50}$
23	SM-23	$Am_3Cf_{100}Fm_5Sm_{100}Sz_{50}Tz_{50}$
24	SM-24	$Am_{30}Cf_{100}Fm_{100}Sm_{100}Sz_{100}Tz_{10}$
25	SM-25	$Am_3Cf_{100}Fm_{100}Sm30Sz_{100}Tz_{50}$
26	SM-26	$Am_3Cf_{50}Fm_{10}Sm_{100}Sz_{30}Tz_3$
27	SM-27	$Am_5Cf_{30}Fm_{100}Sm_{10}Sz_{100}Tz_{30}$
28	SM-28	$Am_5Cf_{100}Fm_{50}Sm_{10}Sz_{50}Tz_{50}$
29	SM-29	$Am_5Cf_{50}Fm_{100}Sm_{10}Sz_{100}Tz_{30}$
30	SM-30	$Am_5Cf_{50}Fm_3Sm_{10}Sz_3Tz_5$
31	SM-31	$Am_{10}Cf_{100}Fm_5Sm30Sz_{50}Tz_{50}$
32	SM-32	$Am_{10}Cf_{100}Fm_5Sm_{100}Sz_{50}Tz_{30}$
33	SM-33	$Am_{10}Cf_{100}Fm_{10}Sm_{30}Sz_{50}Tz_{10}$
34	SM-34	$Am_{10}Cf_{100}Fm_5Sm_{100}Sz_{50}Tz_{30}$
35	SM-35	$Am_5Cf_{10}Fm_5Sm_{30}Sz_{50}Tz_{50}$
36	SM-36	$Am_3Cf_{30}Fm_{10}Sm_{10}Sz_{50}Tz_{50}$
37	SM-37	$Am_5Cf_{100}Fm_{10}Sm_{100}Sz_{50}Tz_{100}$
38	SM-38	$Am_3Cf_{100}Fm_{100}Sm_{50}Sz_{50}Tz_{30}$
39	SM-39	$Am_{10}Cf_{100}F_3S\ m_{30}Sz_{30}Tz_{10}$
40	SM-40	$Am_{10}Cf_{30}Fm_5Sm_{100}Sz_{30}Tz_5$
41	SM-41	$Am_{10}Cf_{30}Fm5Sm_{100}Sz_{30}Tz_5$
42	SM-42	$Am_3Cf_3Fm_3Sm_{10}Sz_3Tz_{10}$
43	SM-43	$Am_{10}Cf_{100}Fm_5Sm_{30}Sz_{30}Tz_{10}$
44	SM-44	$Am_3Cf_{100}Fm_{100}Sm_{50}Sz_{50}Tz_5$
45	SM-45	$Am_5Cf_{100}Fm_{10}Sm_{100}Sz_{50}Tz_{100}$
46	SM-46	$Am_5Cf_{100}Fm_5Sm_{50}Sz_{50}Tz_{10}$
47	SM-47	$Am_5Cf_{100}Fm_5Sm_{30}Sz_{30}Tz_5$
48	SM-48	$Am_{10}Cf_{100}Fm_{10}Sm_{30}Sz_{50}Tz_5$
49	Sm-49	$Am_{10}Cf_{50}Fm_{10}Sm_{100}Sz_{30}Tz_5$
50	SM-50	$Am_3Cf_{100}Fm_{10}Sm_{100}Sz_{30}Tz_{50}$
51	SM-51	$Am_3Cf_{30}Fm_5Sm_{30}Sz_{50}Tz_{50}$

*Resistances were recorded at six differentconcentrations of each antibiotic i.e; 3ug/ml, 5ug/ml, 10ug/ml, 30ug/ml, 50ug/ml & 100ug/ml

**Am = Amikacin
Cf = Cefotaxime
Fm = Fosfomycin
Sm = Streptomycin
Sz = Sulzone
Tz = Tazocin

DISCUSSION

The present study was aimed to find out the drug resistance of different clinical bacterial isolates obtained from different human sources like blood, pus and stool. It was interested to note that all the 51 isolates were found resistant to one or more antibiotics.

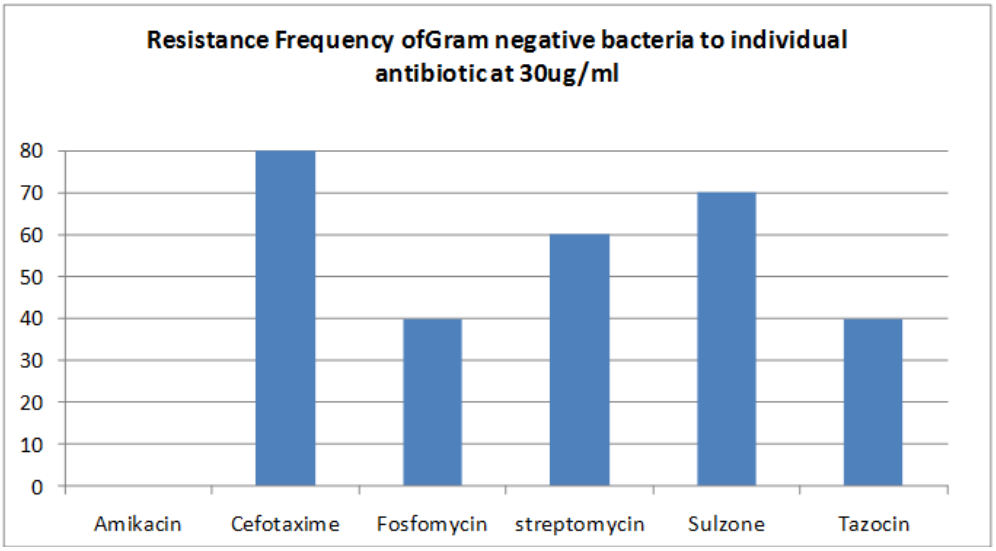

Figure 1.

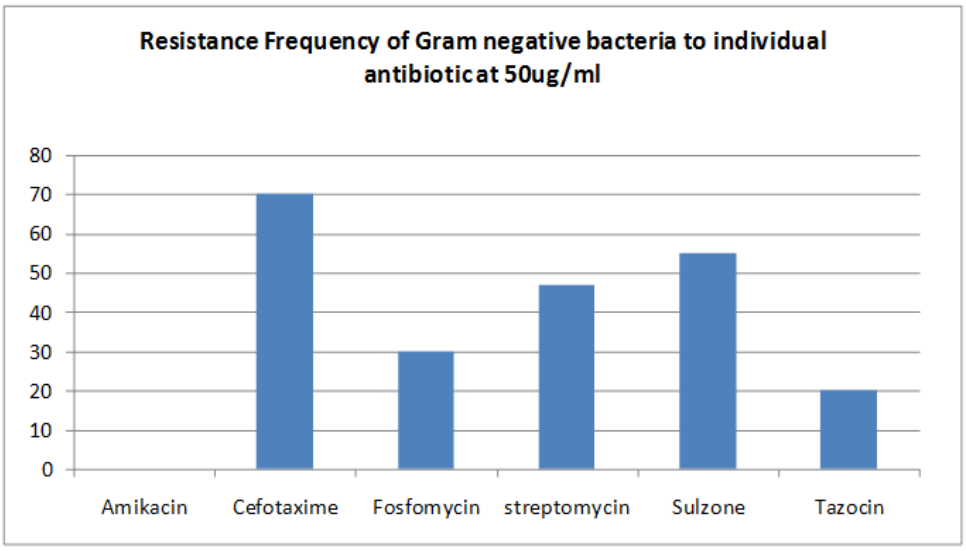

Figure 2.

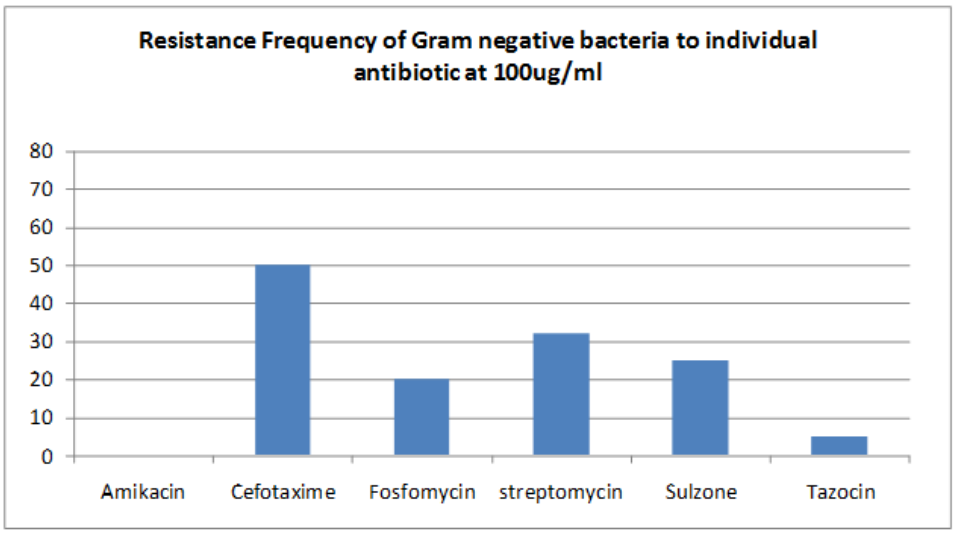

Figure 3.

The organisms not only found resistant but multi drug resistance (resistance to more then one antibiotic) was also prevalent. 43% of the isolates were found to be resistant to one antibiotic, 12 % of isolates were resistant to three antibiotics while 2% were resistant to four antibiotics at a time. The results of this study confirmed the presence of multidrug resistant bacteria in local population of clinical bacteria. Frequency of resistance of bacterial isolates to individual antibiotic was found to be 78% Cefotaxime, 73% for Streptomycin, 70% for Sulzone, 52% for Fosfomycin, 48% for Tazocin and 23% for Amikacin. Most of the isolates were resistant to Cefotaxime and Streptomycin. Amikacin was found to be the most effective antibiotic as no resistance was observed at a concentration of 30ug/ml, 50ug/ml and 100ug/ml. The high frequency of antibiotic resistance among clinical bacteria reflects the indiscriminate use of antibiotics in the treatment of disease. Antimicrobial resistance is a universal problem, constant monitoring of susceptibility pattern is necessary on clinical isolates for selection of appropriate antimicrobial agents for therapy (Pickering, 2004). The study is useful to review the current status of antimicrobial resistance locally and also helpful in minimizing the consequence of drug resistance, limiting the emergence and spread of drug resistant pathogens. Having an awareness of antimicrobial resistance patterns, particularly in hospitals, is crucial for the selection of appropriate antibiotic therapy to improve treatment outcomes, reduce morbidity and mortality, shorten the hospitalization period and consequently reduce the cost of care. The resistances if present on plasmids is more dangerous as it could be transferred conjugally to sensitive strains making them resistant (Desai *et al,.* 1981, Cooper *et al.,* 1984, Fenoll *et al.,* 1987, Sayah *et al.,* 2005, Toroglu *et al.,* 2005) so further investigations about the conjugal transferability of the resistances among bacteria are recommended.

Acknowledgements

The research work was supported by a research grant to Dr. Asma Saeed by the Faculty of Science, University of Karachi, Pakistan.

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