Manarat International University Studies, 3 (1): 177-183, December 2014
ISSN 1815-6754

ISOLATION AND CHARACTERIZATION OF MULTIPLE DRUG RESISTANT BACTERIA FROM UPER RESPIRATORY TRACT INFECTIONS

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ABSTRACT

Overuse of antibiotics has created resistant strains of deadly bacteria which could be a greater threat in poorer nations, owing in part to a lack of regulation on the use of these drugs. Up to 95% of the population in India, Pakistan and Bangladesh carry bacteria that are resistant to β-lactam antibiotics including carbapenems, considered to be antibiotics of 'last resort'. In this study about twenty-five patients having upper respiratory tract infection were screened for multiple drug resistant bacteria. Throat swab, sputum and blood samples of these patients were screened. Two strains of bacteria, Klebsiella, Pneumonia, and Staphylococcus aureus were found to be resistant to eight commonly used antibiotics. Most of the pathogenic bacterial isolate were resistant to three to five antibiotics. The presence of antibiotics resistance genes in multiple numbers in pathogens can cause great health problem for the nation.

Keywords: Antibiotics, Resistance, Bacteria and Plasmids

1. INTRODUCTION

Antibiotics resistance is one of the most serious global threats to the treatment of infectious diseases which is the second largest cause of death worldwide and most significant one for the death of children. Antibiotics are the widely used therapeutics for the treatment of infectious diseases caused by bacteria, but their use has been compromised by the development of resistance to the drugs by the pathogenic bacteria. A number of workers reviewed the present status of antibiotic resistance in human population and pointed out that indiscriminate use of antibiotics lead to the development of resistance against this particular class of drugs by the pathogens. Research work of last few decades established beyond doubt that drug resistance arose from spontaneous or induced genetic mutations or by horizontal gene transfer from other bacteria. The genes for drug resistance have been located on a small circular DNA called transmissible plasmids which are transferred over a wide range of bacteria.

Drug resistance or R plasmids in pathogens play a critical role in designing antibiotic therapy to community. Plasmids carrying multiple markers are called multiple drug resistance markers or MDRs. In clinical conditions pathogenic and commensal bacteria are subject to tremendous selection pressure of high concentrations of antibiotics. As a result these organisms develop antibiotic resistance. Nonjudicious use of antibiotics by clinicians, indiscriminate spray of antibiotics to control pathogens in animal food and feeds and poor management of wastes of antibiotic producing industries lead to the worldwide development of anti-biotics resistance.

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A large number of microbes are associated with upper respiratory tract infections. Among these pathogens *Staphylococcus aureus* and *Klebsiella Pneumonia* are associated with multidrug resistance. These organisms are extremely adaptable to antibiotic pressure. Nosocomial infections caused by these organisms are the leading cause of morbidity and mortality. Multi drug resistant bacteria cause serious community acquired infections which are becoming a serious antibiotic management problem worldwide.

It is a well-established fact that antibiotic is not the drug of choice for the treatment of viral infection. In most of the developing countries physicians prefer to use antibiotics in order to prevent secondary infections. Most of the patients suffering from viral fever prefer to take antibiotics without a prescription. In many instances patients do not complete the prescribed course of antibiotics. As a result antibiotic resistance is increasing and becoming a threat to the health care system of the country. From the 20th to the 21st century antibiotics have been playing critical role in the treatment of infectious diseases and every year newer antibiotics are coming up replacing the older ones but their efficacy is threatened by the emergence of resistant pathogens.

It has been well established that antibiotic resistance property in an organism is a genetic phenomenon which has evolved over the years. It is encoded in the chromosome or plasmid DNA of the bacteria which may be pathogenic or non-pathogenic. This gene or gene family is responsible for drug resistance. It is present in plasmid and transferred horizontally from one bacterium to another. Recent studies indicated that a lot of microbes present in human system have not yet been identified. These commensals present in human system gradually become antibiotic resistance when the Subject takes the drug. These organisms then transfer them to pathogenic organisms either the mechanism of transfer of drug resistance occurs in soil which is a great resort for the pathogenic and non-pathogenic microbes.

A plausible mechanism for the development of the drug resistance has been put forward by. According to this mechanism the pathogenic microbes modify, degrade target molecule, and lowers its concentration inside the cell by increasing efflux or decreasing influx. Recent metagenomic studies points to the fact that sequencing the plasmids and relevant genes might be helpful in designing noble antibiotics to combat the pathogens.

In this respect it may be necessary to know the antibiotic resistant pattern of *Klebciella Pneumoniae* and *Staphylococcus aurius* associated with hospital infections and they easily develop multiple drug resistance and also have very good track record of transferring their resistant property to their neighbors. It is worth to study the plasmid profile of these organisms.

2. OBJECTIVE OF THE STUDY

This study was undertaken in order to find out the prevalence of multiple drugs resistance (MDR) pattern among the pathogens associated with upper respiratory tract infection. The findings may influence the clinicians to use antibiotics very judiciously.

3. METHODS AND MATERIALS

Routine methods and material followed in the diagnostic laboratories were used in this study. In most experiment premade media of Oxoid or Difco Company of USA were used. Throat swabs, sputum and blood samples were collected from the patients attending the out-patient departments of Gono Shastho Nagar Hospital. Samples were collected over a period of six months from thirty patients having upper respiratory tract infections. Most of the patients belonged to low income group. By profession majority of them were rickshaw pullers. Throat swab specimens were collected using a sterile cotton- wool swab and inserted it in a labeled container of Amies Transport Medium.

Sputum for microbiological investigation is collected from the patient in a clean, dry, wide- necked, and leak proof labeled container. Containing nutrient broth and was incubated for six hours. Singe colonies were isolated on nutrient agar plate using serial dilution technique. Those

samples which showed the presence of microbes were transferred into a sterile screw capped bottle containing sterile nutrient broth. Few colonies were gram stained and examination under oil emulsion lense.

A dry — sterilized one time syringe was used to collect 5 ml. venous bloods with all aseptic precautions using separate sterile needle. Blood was introduced aseptically into a blood culture bottle which contains 50ml media containing peptone, dextrose, sodium succinate, sodium lactate, gelatin, sodium carbonate and blue tetrazolium. Growth of the organism is indicated by the change of color. Cultures which showed positive growth were used for the isolation of bacteria using pour plate and streak culture method. For initial isolation the nutrient agar media was used in our study. For the identification of the organisms differential media like McConky, TSA etc were used.

Antibiotic resistance pattern was detected by the disc diffusion techniques. (6) Minimum inhibitory concentration of antibiotics was determined by using 30µg, 50µg, 75µg and 100µg concentration of antibiotics for selective pathogenic strains.

Organisms were identified following the methods described in Bergey's manual (7). All the growth media used in this study were obtained from Sigma. The chemicals used in this study were of analytical grade. Most of the biochemical methods followed in this study as follows: catalase test, Oxidase test, Nitrate test, Coagulate test, Malonate Utilization test, Methyl Red test, Voges–Proskauer test, Kliger's Iron Agar (KIA) test, Motility Indole Urea (MIU) test and Simmons' citrate.

4. RESULTS

Throat swabs, sputum and blood samples were processed and the organisms present in this samples were grown in Luria Broth. The growth of the organisms in each sample was shown in Nutrient agar plate and the number of clones in each sample was counted. Growth could be detected only in the throat swab and sputum samples of some patients. No growth were observed in this samples of twenty patients.

Table no. 1 Growth of Organisms in Different Samples.

Subject No	Throat Swab (CFU/ml)	Sputum (CFU/ml)	Blood (CFU/ml)
1	$2x10^3$	$3X10^3$	–
2	-	$2X10^3$	–
3	$1x10^3$	$4X10^3$	–
4	++	$3X10^3$	+
5	$2X10^3$	$4X10^3$	–
6	$2X10^3$	$2x10^3$	+
7	++	$1X10^3$	–
8	$3X10^3$	$3X10^3$	$2X10^3$
9	–	$1X10^3$	–
10	++	++	–

The results are presented in table 1.growth was reasonable good in the sputum sample followed by throat swab. Growth was very scanty in blood samples. (-) signs indicate no growth while ++ sign shows growth but no discrete colonies could be counted in this samples. No growth could be detected in the blood samples of majority of patients. Growth was reasonably good in the throat swab and sputum of many patients. Only in one or two patients organisms could be detected from the blood samples.

4.1. Identification of the organisms

The organisms were identified by using routine morphological and biochemical tests. The analysis the results shows that majority of the isolates belong to the genus *Klebciella* and *Staphylococcus* and occasionally pseudomonas were detected in some samples. These results were not shown in tabular form because this was isolate at clinical laboratories of different hospitals. The study had serious limitation of sample collection and only thirty samples were collected over a period of six months and out of them twenty samples had no growth profile. Since samples were small in size no attempt to randomization of samples prior to collection were taken. This is a big limitation of this study. Under the circumstances attempts were directed on the isolation and characterization of organisms which had multiple drug resistance attempts. The result5s of multiple drug resistance of *Klebciella Pneumoniae* and *Staphylo coccus aurius* were presented on table 2 and 3.

Table 2: Antibiotic sensitivity of Staphylococcus Aurius

Isolate no	Amoxi-cillin	Erythro-mycin	Cloxa-cillin	Cepha-lexin	Cipro-floxacin	Genta-mycin	Ceftri-axone	Cloram-phenicol	Oflo-xacin	Kana-mycin	Azithro-mycin
1	+	+	+	+	+	–	+	+	+	–	–
3	+	–	+	+	+	–	–	+	+	–	–
4	+	+	+	+	+	–	+	+	+	–	–
5	+	–	+	–	+	–	+	+	–	–	–
6	+	+	+	+	+	–	+	+	+	–	–
8	+	+	+	+	+	–	–	–	–	–	–
10	+	–	+	–	–	–	–	–	–	–	–

Table 3. Resistance of Klebciella Pneumoniaeagains antibiotics

Isolate no	Amoxi-cillin	Erythro-mycin	Cloxa-cillin	Cepha-lexin	Cipro-floxacin	Genta-mycin	Ceftri-axone	Cloram-phenicol	Oflo-xacin	Kana-mycin	Azithro-mycin
1	+	_	+	+	_	_	+	_	_	_	_
3	+	_	_	_	+	_	_	_	_	_	_
4	+	+	_	_	+	_	+	+	+	_	_
5	+	+	+	+	+	_	+	+	+	_	_
6	+	_	_	_	_	_	_	_	+	_	_
8	+	+	+	+	_	_	+	+	+	_	_
10	+	+	+	_	_	_	_	_	_	_	_

+=Resistant And _ Sensitive

A close analysis of the results presented in table 2 and 3 show that all the organisms were resistant to more than one antibiotic. Some of them are resistant to seven to eight antibiotics. The minimum concentration of the antibiotic used to test the sensitivity of the organisms was 30µg/ml. most th e anti sensitive to the commonly used antibiotics such as amoxicillin erythromycin, cloxacilin, cephalexin, ciprofloxacin, cloramphenicol, ceftriaxone and ofloxacin. And they are sensitive to azithromycin, gentamycin and kenamycins which are expensive drug for poor patients. MIC of these antibiotics has been determined using 50µg/ml 75µg/ml and 100µg/ml. and the results have been shown in table 4.

Table: 4 MIC of Klebciella Pneumoniaeagains and Staphylococcus Aurius

Isolate no	Amoxi-cillin	Erythro-mycin	Cloxa-cillin	Cepha-lexin	Cipro-floxacin	Genta-mycin	Ceftri-axone	Cloram-phenicol	Oflo-xacin	Kana-mycin	Azithro-mycin
T	+	+	+	+	+	I	+	+	+	I	I
S	+	+	+	+	+	I	+	+	+	I	I

Mic of the antibiotics was determined against *Klebciella pneumoniaeagains* and *Staphylococcus aurius*. The concentration antibiotics used were 30, 50, 75 and 100 µg/ml. the organisms were resistance to all this concentration of antibiotics. Therefore concentrations below 100 µg/ml were not shown in the table. Only the results for 100 µg/ml antibiotics were shown in the table. This results indicate that the organisms are resistant to very high concentration of antibiotics.

5. CONCLUSION AND RECOMMENDATION

Multidrug resistance is alarming because it can be transferred horizontally from *Klebciella pneumonia* and *Staphylococcus aurius to E.coli* and other *enterobacteriaceae.* As a result infectious diseases may become uncontrollable in economically backward countries. The multidrug resistant plasmids should be isolated and the genes present in them should b analyzed by using metagenomic approaches. This may help in designing new antibiotics for multidrug resistant organisms.

6. ACKNOWLEDGEMENT

We gratefully acknowledge the assistance of Kazi Afekul Islam, Jr. Consultant, Biochemistry Division, Popular Diagnostic Laboratory, Mirpur and Ms. Anwara Begum of Gono Shastha Nagar Hospital for generously providing us with clinical samples. We also thank MS Shahin Rahman, Lab Tecnician Department of Pharmacy Manarat International University for her technical help.

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