

Original Research Article

# Molecular characterization and antibiotic resistance of bacterial pathogens from respiratory tract infection

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## Abstract

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In the present study the bacteria were isolated from sputum samples of patients suffering from respiratory tract infection. These virulent bacteria were checked for antibiotic resistance. All antibiotic resistant bacteria were biochemically characterized at genus level (*Streptococcus* sp. and *Pseudomonas* sp.). Three bacterial strains were confirmed by ribotyping at species level named *Bacillus cereus*, *Klebsiella pneumoniae* and *Pseudomonas putida*. The Results indicated that all the strains were highly resistant against Cloxacillin, Amoxyllin/Clavulanic acid, Oxacillin, Cefepime and Ampicillin. *Streptococcus* sp. and *Pseudomonas* sp. were also having resistance potential against Penicillin. Penicillin is not effective against *Pseudomonas* sp. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were also checked against azithromycine, doxycycline, cephalixin, cefepime, tetracycline, gentamicin and amikacin for all bacterial species. The highest MIC of *Bacillus cereus* was observed as 78.1µg/ml against Gentamicin. Like *Bacillus cereus*, *Klebsiella pneumoniae* was also having highest MIC 78.1µg/ml against Gentamicin. 48.8µg/ml tetracycline was examined the higher MIC for *Pseudomonas putida*. The MBC against antibiotics were also observed for bacterial strains and most of these MIC values were unchanged while others were high. From this study, it was concluded that the antibiotic resistant bacteria were progressively increased due to continuous exposure to same antibiotics. This increase of MIC and MBC of various antibiotics against bacterial species was seemed due to their higher drug resistance potential.

**Key words:** Antibiotics, *Bacillus cereus*, *Klebsiella pneumoniae*, *Pseudomonas putida*, Respiratory tract infection, Ribotyping

## INTRODUCTION

Any infections of sinuses, throat, airways or lungs are collectively called respiratory tract infection (RTI). RTI is commonly found in children than in adults as they have not yet developed immunity against many of these infectious agents Frankard *et al.*, 2004). Globally, five million deaths are estimated every year due to RTIs from which 10 to 15 percent deaths are caused by lower respiratory tract infections. There are many infectious diseases of

upper or lower respiratory tract, either in acute or chronic form. RTIs are generally divided as infections of upper and lower respiratory tract (Farrell, 2004). Upper respiratory tract infections (URTIs) affect nose, sinuses, throat and include common cold, acute otitis, rhinosinusitis, pharyngitis/tonsillitis, rhinitis and laryngitis whereas airways and lungs are affected in lower respiratory tract infections (LRTIs) which include pneumonia, acute trachei

tis, bronchitis and bronchiolitis (Riley and Riley, 2003). The lower airways diseases such as bronchiectasis and COPD, the isolation of bacteria such as *Haemophilus influenzae* and *Pseudomonas* species in sputum samples by culture is not uncommon. While these pathogens are often associated with exacerbations, they are also often present during stable phase of the airways disease indicating chronic colonization. The isolation of bacterial pathogens in chronic asthma by culture remains understudied. In a report, 27% of asthmatic patients presenting with an exacerbation of asthma had bacteria in sputum with *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Moraxella catarrhalis* and *Haemophilus influenzae* (Cazzol *et al.*, 1991).

An X-Ray analysis of thoracic region suggests respiratory infection while sputum culture characterizes the responsible microbial agent. Viruses are a major cause of such infections but bacterial infections are also common. Culture of sputum shows variety of pathogenic bacterial strains responsible for some particular diseases like *Streptococcus pneumoniae* is the most effective pathogen causing bacterial pneumonia, other pathogenic bacteria include *Haemophilus influenzae*, *Staphylococcus aureus*, *Enterobacter sp.*, *E. coli*, *Klebsiella pneumoniae*, *Legionella pneumophila*, and *Mycobacterium tuberculosis*. Various pseudomonas species are also pathogenic such as *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas savastanoi* that cause infection in urinary system, respiratory system and nervous system (Yang *et al.*, 2004).

Antibiotics are used against infectious diseases caused by bacteria while have no effect on viruses. Antibiotics interrupt bacterial growth by hindering one of its vital processes necessary for the survival of bacteria, such as synthesis of cell wall. Antimicrobial resistance is not modern because the various drug resistance micro-organism were found throughout the world but the breadth of drug resistance in micro-organisms are unpredicted and mounting (Levy, 2003).

In present study, main focus was to screen pathogenic bacteria from respiratory tract infections. Also to check the antibiotic resistance of these bacterial pathogens. MIC and MBC of antibiotics against these pathogens were also studied.

## MATERIALS AND METHODS

### Sample Collection

Study samples (sputum) were collected from outdoor and indoor patients suffering from respiratory tract infection by coughing out sputum sample from Ganga Ram Hospital, Lahore, Pakistan. Sputum sample was taken early morning before taking meal in sterilized eppendorf to avoid contamination (Paggiaro *et al.*, 2002). Sputum samples

were transferred to the microbiology laboratory, G.C. University Lahore, Pakistan.

### Bacterial Isolation

Sputum sample was inoculated on MacConkey agar, Blood agar and Chocolate agar with sterile platinum wire loop and incubated at 37°C for 18-24hours. Growths of various microbial colonies were seen, after overnight incubation. The morphology of different colonies were isolated inoculating loop and then streaked on fresh agar plates to get pure culture of bacterial strains.

### Morphological and Biochemical Analysis

The isolated bacteria were characterized by performing various morphological and biochemical tests. Gram Staining, Endospore Staining and Motility test were performed for morphological analysis, while Catalase, Urease, Gelatin, Litmus milk, Triple sugar iron (TSI), Citrate, Oxidase, Indole, Hydrogen sulphide (H<sub>2</sub>S) production, Blood agar and MacConkey agar tests were selected for biochemical analysis of bacterial isolates. All tests were performed according to Bergey's Manual of Systematic Bacteriology. In this work, all the antibiotic resistance bacterial isolates were biochemically characterized by utilizing Bergey's manual key and three of them confirmed by ribotyping include *Bacillus cereus*, *Klebsiella pneumoniae* and *Pseudomonas putida*. Ribotyping technique has been commonly utilized for identification and confirmation of bacterial species among vast diversity of bacteria. This technique includes DNA isolation (Sambrook *et al.*, 1989), gel electrophoresis, PCR product base sequencing (Katarzyna and Piotr, 2008).

### Assessment of Antibiotic Resistant Bacterial Isolates

The resistance or sensitivity of bacterial isolates was checked against a group of broad-spectrum antibiotics (AZM15mcg, DOX30mcg, CLX5mcg, CEP30mcg, OXA10mcg, CEF30mcg, AMP10mcg, TET30mcg and PEN10mcg) by following Kirby-Bauer disc diffusion method. Growth inhibition zones appeared after overnight incubation of bacterial strains with antibiotic discs and calculated.

### Molecular Characterization

Chloroform/Phenol DNA Extraction method was used for the extraction of bacterial genomic DNA with minor adjustments (Sambrook, *et al.*, 1989). For gene amplification, Polymerase Chain Reaction (PCR) was performed

to amplify the specific sequence of DNA. The aim of PCR was to obtain numerous copies of specific region of ribosomal RNA gene. Invitrogen Platinum® PCR SuperMix HighFidelity (Cat. No. 12532\_016, size: 100 reactions) was used for gene amplification which had Recombinant Taq DNA Polymerase, Taq antibody, 66mM tris-SO<sub>4</sub> (pH 8.9), 19.8mM (NH<sub>4</sub>)SO<sub>4</sub>, 2.4mM MgSO<sub>4</sub>, 220µM dNTPs, and stabilizer. This mixture was helpful for the amplification of large ranged DNA template sized upto 15kb genomic DNA. For 16S ribosomal RNA gene amplification universal primers were used (i.e. 27F sequence 5'-AGAGTTTGATCCTGGCTCAG-3' and 1522R sequence 5'-AAGGAGGTGATCCAGCCGCA-3'). Thermocycler (PROGENE, Tech) was adjusted for initial denaturation at 94°C for 2 minutes, melting at 94°C and annealing at 55°C, primer extension at 72°C, each with 60 seconds, and 35 cycles of PCR were performed. Final extension was done at 72°C for 5 minutes and end storage was adjusted to 4°C for maximum period of time. Gel electrophoresis was performed for the quantification and separation of particular DNA band. For gene clean, Invitrogen DNA by life technologies, pure link™, quick gel extraction kit was used.

### Sequencing PCR-Fragment (Purified)

After Gene Clean, PCR-Fragments were carried to Centre for Advanced Molecular Biology (CAMB), University Of Punjab, Lahore for sequencing.

### Sequence Analysis

Sequence results from CAMB were analyzed using BLAST, National Centre for Biotechnology Information (NCBI) for molecular characterization. The entire genome shotgun sequence was applied for analysis.

### Measurement of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration was observed in which minimum amount of antibiotics that can inhibit the visible growth of microbial agents are calculated (Andrews, 2006). Bacteria were inoculated in broth culture and incubated at 37°C overnight. The culture was diluted to prepare inoculums of about 10<sup>5</sup>-10<sup>6</sup> colony forming unit in each milliliter.

### Method of Antibiotic Dilution

Stock solutions of antibiotics were prepared by dissolving a considerable amount of antibiotic in respective solvent

to get stock solution. Two fold dilution methods were utilized to obtain various dilute concentrations of each antibiotic as indicated in table 3.2. 17 autoclaved glass test tubes were placed in a rack and then 15 test tubes were labeled by numbers from 1 to 15. However, the first one was labeled as AC (Antibiotic Control) and last one mentioned as GC (Growth Control). One ml of nutrient broth was poured in each glass tube and then one ml of antibiotic solution was mixed in all test tubes except GC tube. After mixing, 1ml mixture was transferred from tube number one to tube number 2 by using micropipette and sterile tips. Next, the same method was repeated to transfer one ml media from next to next till tube number 15 by using new tip for each dilution. At the end, one ml of mixture from tube number 15 was removed and discarded. The G.C tube served as growth control because it received no antibacterial agent while first tube consider as Antibiotic Control. After it, one ml of broth culture of particular microbial isolate was inoculated in each tube except A.C tube. Thus, the final concentration of antibiotic in these test tubes was diluted to half of the initial concentration because of mixing of equal volume of inoculums in Broth and shown in table 3.3 (a) and (b). Then tubes were incubated in incubator at 37°C for overnight. Next day, the visible growth (i.e. turbidity) in the tubes was observed as bacterial growth and the tubes showed no turbidity considered as MIC for the respective antibiotic (Kowser and Fatema, 2009).

### Minimum Bactericidal Concentration (MBC) Measurement

A nutrient agar plate was prepared to find MBC of each antibiotic. After it, fifty micro liter inoculum was taken with the help of micropipette every time with new sterile tip from each test tube that showed minimum inhibitory concentration as well as the lower concentration. Then, the inoculums was poured and spread into nutrient agar plates. The plates were placed in incubator at 37°C overnight. Next day, MBC was observed as a lowest concentration at which no bacterial growth was found on agar plates (Betts, *et al.*, 2012).

## RESULTS

Gram staining showed that MS1 and MS2 antibiotic resistant bacteria were Gram positive. These test also revealed that MS1 had spores while MS1, MS3 and MS5 were motile as indicated in table 1.

### Assessment of Antibiotic Resistant Bacterial Isolates

The Antibiotic sensitivity test indicated that MS1 (*Bacillus*

**Table 1.** Morphological traits of bacterial isolates from respiratory tract infection (Sputum)

Bacterial Isolates	Morphological tests of bacterial isolates		
	Gram staining	Endospore staining	Motility reaction
MS1	Gram +ve	Spore (+ve)	Motile
MS2	Gram +ve	Lack spore (-ve)	None motile
MS3	Gram -ve	Lack spore (-ve)	Motile
MS4	Gram -ve	Lack spore (-ve)	None motile
MS5	Gram -ve	Lack spore (-ve)	Motile

**Table 2.** Biochemical characterization of bacterial isolates

Biochemical tests	Bacterial isolates				
	MS1	MS2	MS3	MS4	MS5
Catalase Test	+ve	-ve	-ve	+ve	-ve
Urease Test	-ve	-ve	+ve	+ve	+ve
Gelatin Test	+ve	+ve	+ve	+ve	+ve
Litmus Milk test	+ve	+ve	+ve	+ve	-ve
Triple Sugar Iron Test	+ve	+ve	-ve	-ve	+ve
Citrate Test	+ve	+ve	+ve	+ve	+ve
Oxidase Test	-ve	-ve	+ve	-ve	+ve
Indole Test	-ve	+ve	-ve	-ve	-ve
Hydrogen sulphideproduction Test	-ve	-ve	+ve	-ve	+ve
Blood Agar Test	+ve	+ve	+ve	+ve	+ve
MacConkey Agar Test	-ve	-ve	-ve	+ve	-ve
<b>Carbohydrate Fermentation Test</b>					
Glucose	A (+ve)	A(-ve)	A(-ve)	A/G (+ve)	A(-ve)
Sucrose	A (+ve)	A/G (+ve)	A(-ve)	A/G (+ve)	A(-ve)
Lactose	A/G (-ve)	A/G(+ve)	A(-ve)	A (+ve)	A(-ve)
<b>Methyle Red and Voges Proskuer Test</b>					
Methyle Red	-ve	-ve	-ve	-ve	-ve
Voges Proskuer	+ve	+ve	-ve	+ve	-ve
<b>Bacterial Genus</b>	<i>Bacillus</i> sp.	<i>Streptococcus</i> sp.	<i>Pseudomonas</i> sp.	<i>Klebsiella</i> sp.	<i>Pseudomonas</i> sp.

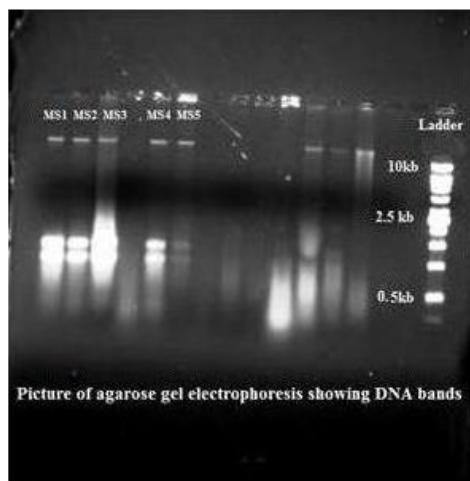
**Table 3.** Antibiogram of antibiotics was shown against particular bacterial isolates

Bacterial isolates	Antibiogram
MS1	AZM <sup>S</sup> , DOX <sup>S</sup> , CLO <sup>H</sup> , CEP <sup>S</sup> , OXA <sup>R</sup> , CEF <sup>S</sup> , AMP <sup>H</sup> , TET <sup>S</sup> , PEN <sup>S</sup>
MS2	AZM <sup>S</sup> , DOX <sup>S</sup> , CLO <sup>R</sup> , CEP <sup>S</sup> , OXA <sup>R</sup> , CEF <sup>S</sup> , AMP <sup>R</sup> , TET <sup>S</sup> , PEN <sup>R</sup>
MS3	AZM <sup>S</sup> , DOX <sup>S</sup> , CLO <sup>H</sup> , CEP <sup>H</sup> , OXA <sup>H</sup> , CEF <sup>S</sup> , AMP <sup>H</sup> , TET <sup>S</sup> , PEN <sup>H</sup>
MS4	AZM <sup>S</sup> , DOX <sup>S</sup> , CLO <sup>R</sup> , CEP <sup>S</sup> , OXA <sup>R</sup> , CEF <sup>R</sup> , AMP <sup>R</sup> , TET <sup>S</sup> , PEN <sup>S</sup>
MS5	AZM <sup>S</sup> , DOX <sup>S</sup> , CLO <sup>H</sup> , CEP <sup>S</sup> , OXA <sup>H</sup> , CEF <sup>S</sup> , AMP <sup>H</sup> , TET <sup>S</sup> , PEN <sup>H</sup>

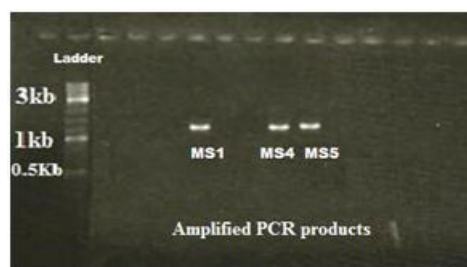
**Note:** 'S' stands for Sensitive while 'R' for Resistance, AZM (Azithromycin), DOX (Doxycycline), CLO (Cloxacillin), CEP (Cephalexin), OXA (Oxacillin), CEF (Cefepime), AMP (Ampicillin), TET (Tetracycline), PEN (Penicillin)

*cereus*), MS2 (*Streptococcus* sp.), MS3 (*Pseudomonas* sp.), MS4 (*Klebsiella pneumoniae*) and MS5 (*Pseudomonas putida*) were sensitive to Azithromycin (AZM15), Doxycycline (DOX30) and Tetracycline (TET30). MS1 and MS4 bacterial strains were also sensitive to antibiotics Cephalexin (CEP30) and penicillin (PEN10), While MS2 and MS4 to Cephalexin (CEP30). All the concerned strains including *Bacillus cereus*, *Klebsiella pneumoniae*

and *Pseudomonas putida* and *Streptococcus* sp. showed complete resistance against Cloxacillin (CLO5), Amoxyl- lin/Clavulanic acid (AMC30), Oxacillin (OXA10), Cefepime (CEF30) and Ampicillin (AMP10). MS2 were again showed resistance to Penicillin (PEN10) while MS3 were resistance Cephalexin (CEP30) and Penicillin (PEN10) as represented in table 3.



**Figure 1.** Isolated DNA bands of bacterial isolates after agarose gel electrophoresis



**Figure 2.** Amplified PCR products (16s rDNA) of MS1 (*Bacillus cereus*), MS4 (*Klebsiella pneumoniae*) and MS5 (*Pseudomonas putida*) bacterial isolates

**Table 4.** Molecular characterization of antibiotic resistance bacterial isolates

Bacterial isolate	Description
MS1	<i>Bacillus cereus</i>
MS4	<i>Klebsiella pneumoniae</i>
MS5	<i>Pseudomonas putida</i>

### Molecular Characterization of Antibiotic Resistant Bacteria

The extracted chromosomal DNA of antibiotic resistance bacterial isolates revealed that chloro form/phenol DNA extraction method was highly suitable and effective because it gave high DNA concentrations as shown in figure 1. The Invitrogen Platinum® superMix kit and both the primers were observed to be reliable and effective for target DNA amplification as shown in figure 2. The 16S rRNA gene sequence analysis of antibiotic resistance bacterial isolate indicated that MS1 bacterial strain was 99% similar to species *Bacillus cereus* while MS4 was

98% related to bacterial species *Klebsiella pneumoniae*. Similarly MS5 shared close resemblance with *Pseudomonas putida* species as shown in table 4

### Optimum Growth Conditions

The results indicated that the optimum temperature was 37°C for molecularly identified bacteria such as *Bacillus cereus*, *Klebsiella pneumoniae* and *Pseudomonas putida* (Figure 3). Similarly the optimum pH ranged between 7-8 pH for same bacterial species (Figure 4).

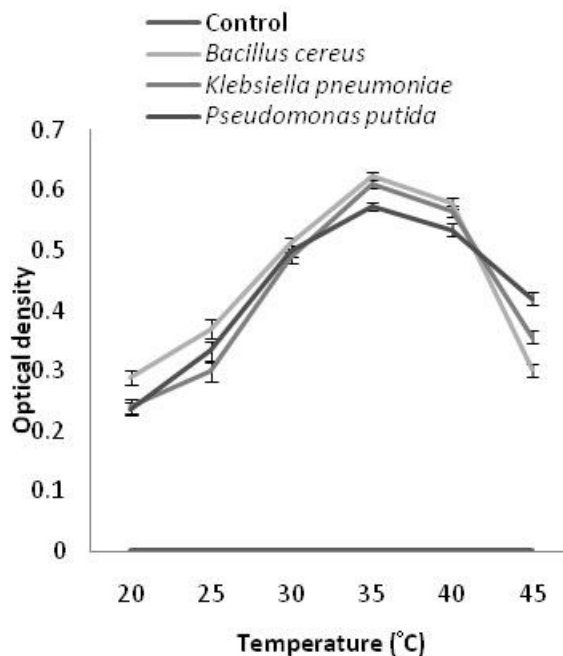


Figure 3. Optimum temperature for bacterial growth

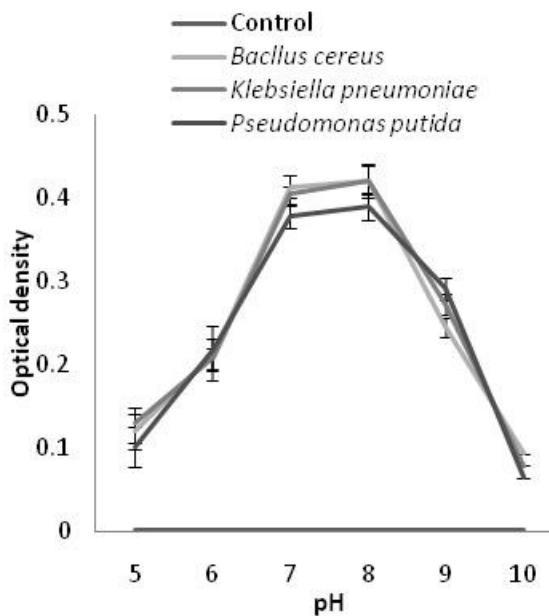
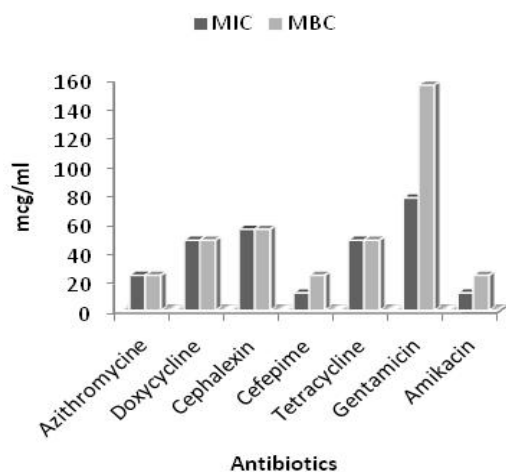


Figure 4. Optimum pH for bacterial growth

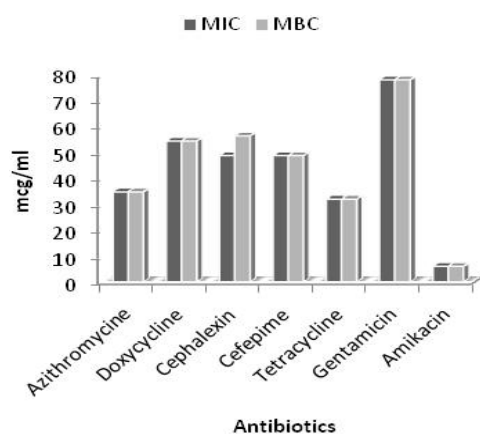
### Comparison of MICs and MBCs

The values of MIC and MBC of respective antibiotics were compared for each bacterial isolate. The MBC of Cefepime, Gentamicin and Amikacin were higher than MIC against *Bacillus cereus*. For *Bacillus cereus*, the values of MICs and MBCs were same for Azithromycine

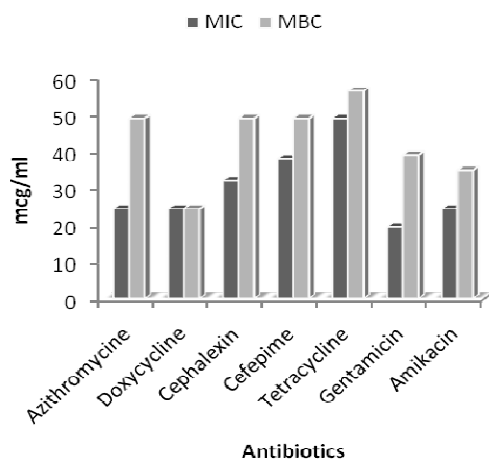
(24.4µg/ml), Doxycycline (48.8µg/ml), Cephalexin (56.4µg/ml) and Tetracycline (4.8.8µg/ml) (Figure 5). The MBC of Cephalexin antibiotic was high than MIC for *Klebsiella pneumoniae*. But MIC and MBC of antibiotics like Azithromycine, Doxycycline, Cefepime, Tetracycline, Gentamicin and Amikacin were observed similar that was 34.8µg/ml, 54.4µg/ml, 48.8µg/ml, 32µg/ml, 78.1µg/ml and



**Figure 5.** Comparison of MIC and MBC values of various antibiotics against MS1 (*Bacillus cereus*) bacterial isolate



**Figure 6.** Comparison of MIC and MBC values of various antibiotics against MS4 (*Klebsiella pneumoniae*) bacterial isolate



**Figure 7.** Comparison of MIC and MBC values of various antibiotics against MS5 (*Pseudomonas putida*) bacterial isolate

6µg/ml respectively, as indicated in Figure 6. The values of MBC of Azithromycin, Cephalexin, Cefepime, Tetracycline, Gentamicin and Amikacin were higher than MICs against *Pseudomonas putida* but it had similar MBC and MIC for Doxycycline (24.4µg/ml) as shown in Figure 7.

## DISCUSSION

In this research work, the pathogenic antibiotic resistant bacterial isolates, such *Bacillus cereus*, *Streptococcus* sp., *Pseudomonas* sp., *Klebsiella pneumoniae* and *Pseudomonas putida*, were isolated from sputum samples that caused infectious diseases in lungs. These results have also been reported by Ravi *et al.*, (2010).

The genetic evaluation of bacterial chromosomal DNA by ribotyping has now been additional screening power over morphological and biochemical characterization. Moreover, it also differentiates between members of the same species and classified at sub-species level.

The optimum growth temperature for molecularly identified antibiotic resistance bacterial isolates was 37°C and optimum pH was ranged 7-8. The results regarding to these growth conditions were well documented by various researchers (Joseph, 2008).

In the present work, it was observed that *Bacillus cereus* isolates were quite susceptible to certain β-lactams and macrolids such as penicillin, Cephalexin, Azithromycin, Doxycycline and Tetracycline. Moreover, *B. cereus* showed resistance against Cloxacillin, Amoxyllin/Clavulanic acid, Oxacillin, Cefepime and Ampicillin. Similarly, literature revealed that various *Bacillus cereus* strains were sensitive to Doxycycline and Tetracycline (Ravi *et al.*, 2010). Other authors also documented in their studies that *B. cereus* isolates had become resistant against certain β-lactam antibiotics like Ampicillin, Cephalotin, Oxacillin and Streptomycin (Yehia *et al.*, 2008; Behera, 2010).

In this study, the *Streptococcus* sp. isolates were confirmed as pathogenic, Gram positive, spherical and non motile antibiotic resistant bacteremia. These results were comparable to the publication of "The center for food security and public health" in 2005. Chiou and Hsieh (2003) were also investigated that *Streptococcus* sp. was a serious infectious agent of bacterial meningitis, sinusitis, pneumonia and otitis media in the children.

The results regarding to drug resistance in recent work, the *Streptococcus* sp. were showed resistance to Ampicillin, Amoxyllin/Clavulanic acid, Cloxacillin, Cefepime, Oxacillin and Penicillin. Similarly, Multi-Drug resistant *Streptococcus* sp. had long been reported as major contributor of respiratory infections in young and adults worldwide. But the antibiotic drug resistance has increased tremendously in *Streptococcus* sp. and become a big threat to health issues (Farrell and Felmingham, 2004). Especially, these occur for macrolides and β-

lactams which are prescribed as first-line antimicrobial agents against streptococcal infections in France and Asia (Song *et al.*, 2004).

In present work, *Pseudomonas* sp. was proved as Gram negative, motile but non-spore forming and highly virulent bacterial isolate. According to Christopher *et al.* (2011), *Pseudomonas* sp. was frequently involve in respiratory tract infection and can be isolated from sputum samples.

Like other bacterial strains, *Pseudomonas* sp. was observed resistant to Cloxacillin, Amoxyllin/Clavulanic acid, Oxacillin, Cefepime, Ampicillin, Cephalexin and Penicillin in the present study as reported by Marchandin *et al.* (2000). This multiple antibiotic resistance was usually concerned with the synthesis of β-lactamases enzymes (Senda *et al.*, 1996). *Pseudomonas* sp. was also reported by several authors for transferring antibiotic resistance capability to other bacterial species because their plasmid contains drug resistance genes (Agodi *et al.*, 1995).

In this research work, it was also investigated that *Klebsiella pneumoniae* were Gram negative, rod shape, non motile and lack of spores forming capability (Amin *et al.*, 2009). Many bacterial strains of *Klebsiella* sp. were virulent that causes infectious diseases in man and frequently treated by prescribing β-lactams. The basic antibiotic Drug-Resistance mechanism in *Klebsiella pneumoniae* to broad spectrum cephalosporin and penicillin depend on the synthesis of enzymes β-lactamases, because of this resistance, alternative antibiotics were used such as quinolone and aminoglycosides for treatment. The results of this study also revealed that the reproductive capabilities of genus *Klebsiella* isolates were not inhibited by Cloxacillin, Amoxyllin/Clavulanic acid, Oxacillin, Cefepime and Ampicillin because they were highly resistance to these antimicrobial agents (Amin *et al.*, 2009).

Zuberbuhler and Carifi (2012) were, similar to the results of present work, investigated that *Pseudomonas putida* was pathogenic, Gram negative, motile and non-spore forming antibiotic resistance microbial strain. Previously, Blazevic *et al.* (1973) believed that *Pseudomonas putida* bacterial species were less virulent than other *Pseudomonas* sp. and had very less importance in clinical trials. According to Seong *et al.* (2012), the death rate due to pneumonia caused by *P. putida* isolate was increased from thirteen percent (13%) to forty percent (40%). Instead of all these facts, as *P. putida* were less virulent but this microbe possessed high antibiotic susceptibility compared with *Pseudomonas* sp. So, it was cleared that its effect on increased mortality rate is due to its high resistance against antimicrobial drugs (Almuzara *et al.*, 2007). From this work, it was clarified that *P. putida* isolates were showed resistance against Cloxacillin, Amoxyllin/Clavulanic acid, Oxacillin, Cefepime and Ampicillin.

In this present research, the minimum inhibitory concentrations of antibiotics include Azithromycine, Doxycyc

line, Cephalexin, Cefepime, Tetracycline, Gentamicin, Amikacin for *Bacillus cereus* and *Klebsiella pneumoniae* were observed high as compared to the work of Shadlia et al. (2008). Previous literature revealed that MIC and MBC of bacterial species were observed in which some MIC and MBC values were same while in some cases MBC was higher than MIC for *Bacillus cereus*, *Klebsiella pneumoniae* and *Pseudomonas putida* (Lorenzo et al., 2002).

This study gave a valuable knowledge about the drug resistance capability of *Bacillus cereus*, *Klebsiella pneumoniae* and *Pseudomonas putida* against various antibiotics. From this study, it was also concluded that MBC and MIC values varies among bacterial species against particular antibiotic (Adegoke et al., 2010).

## CONCLUSION

From present work it was concluded that the rate of respiratory infections is increased due to bacterial infections. Also antibiotic resistance in bacteria has progressively increased due to misuse and overuse of antibiotics. In this study, the increase in value of MIC and MBC of various antibiotics against bacterial species reflected the higher drug resistance potential of these bacterial pathogens. So there is need to control the bacterial contamination and improve hygienic conditions especially in countries like Pakistan where health problems are major issue.

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## Competing Interests

Authors have declared that no competing interests exist.

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