Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7671

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2019) *7*(4), 54-64

Research Article

Indian Journal of Pure & Applied Biosciences

Current Status of Multi Drug Resistance of Bacillus Species from Clinical Sources

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Received: 25. 06.2019 | Revised: 29.07.2019 | Accepted: 6.08.2019

ABSTRACT

Bacillus species are found anywhere in environment. Due to unnecessary use of antibiotics many bacteria are becoming resistant to antibiotics. Many bacterial strains become resistant due to the unnecessary use of antibiotics. When bacteria become resistant the antibiotic loses their ability for the elimination of bacteria from the body of infected person. 110 clinical samples were collected from different hospitals of Lahore. These samples were purified. After purification 20 Bacillus bacteria were isolated by using differential media MSA. Structures were examined under microscope after examination three bacillus species (B. subtillis, B. licheniformis and B. cereus) were found. For further confirmation biochemical tests were performed. Antimicrobial susceptibility test was performed at the end to check the bacterial susceptibility against antibiotics. Ten different antibiotics were used by disc diffusion method. Antibiotics were streptomycin, clindamycin, gentamicin, ampicillin, tetracycline, azithromycin, vancomycin, chloramphenicol, oxacillin and amoxicillin. 95% of the resistance was shown against oxacillin and 95% against ampicillin. 95% sensitivity was shown by streptomycin. 90% sensitivity was shown by tetracycline and gentamicin. 100% sensitivity was shown by three antibiotics against B. licheniformis which were streptomycin, tetracycline and gentamicin while no sensitivity was given by ampicillin, amoxicillin, oxacillin and clindamycin. 100% resistance was shown by oxacillin against B. licheniformis. No resistance was seen in the case of streptomycin, tetracycline and gentamicin. Maximum sensitivity was shown by streptomycin, tetracycline and gentamicin against B. subtilis. Minimum sensitivity was given by clindamycin. 100% resistance was shown by oxacillin and 89% resistance was given by amoxicillin. B. cereus is shown different percentages of resistance and sensitivity against ten different drugs. Maximum sensitivity was shown for streptomycin, tetracycline and gentamicin while maximum resistance was shown against ampicillin and oxacillin.

Keywords: *Antibiotics, Resistant, Spores, Clinical samples, Pathogenic, Disease.*

INTRODUCTION

The genus Bacillus is one of the largest genus finds everywhere simultaneously. It has great phenotypic diversity. This genus comprises of 268 sp and 7 sub *sp* almost all the sp are found in environment and considered as laboratory contaminants but few sp like *B. anthracis* and *B. cereus* cause infections in humans.

Cite this article: Mahmood, A., Sharif, M., Ahmad, Q., Shabbir, S., Afzal, K., & Mahmood, R. (2019). Current Status of Multi Drug Resistance of Bacillus Species from Clinical Sources, *Ind. J. Pure App. Biosci.* *7*(4), 54-64. doi: http://dx.doi.org/10.18782/2320-7051.7671

B. anthracis causes anthrax and *B. cereus* causes foodborne illness. Bacillus bacteria are Gram positive bacteria. They have a rod shape structure and usually occur in pairs or chains. Their ends are rounded or square having a single endospore. Endospores also varies in shape some are oval, some are round and others are cylindrical (Barbosa, & Levy, 2000). On the basis of structure of spore and sporangium these bacteria are classified into three groups, Group1, group2 and group3. Group1 gram positive rods contain central or terminal, ellipsoidal or cylindrical spores. Sporangium is not swollen by these spores. Group1 is further classified into two subgroups which are large cell sub group and small cell subgroup. Large cell subgroup contains *B. anthracis, B. cereus, B. mycoides, B. thuringiensis, and B. megaterium.* Small cell subgroup contains *B. pumilus, B. subtilis and B. licheniformis*.Group2 *Bacillus* bacteria have central, ellipsoidal spores and they have swollen sporangia. Sp include in this group are *B. circulan, B. coagulans, B. alvei, B. brevis and B. macerans* Group3 have swollen sporangia having terminal or sub terminal spores. *B. sphaericus* includes in this *Bacillus sp* cause different types of infections in humans like *B. cereus* causes infections of eyes e.g. conjunctivitis, panophthalmitis, keratitis, iridocyclitis, dacryocystitis, and orbital abscess. Most serious eyes infection is panophthalmitis. *B. cereus* also causes central nervous system infections, wound and gangrenous infections, miscellaneous infections, infections in genital tract of female and food poisoning. Toxins produced by *B. cereus* also cause different types of infections. *B. licheniformis* causes opthalmitis, corneal ulcer, and food poisoning. *B. subtilis, B. brevis and B. coagulans cause food poisoning. B. macerans* causes wound infection, *B. pumilus* causes pustules and rectal fistula infections, *B. alvei* causes meningitis and *B. sphaericus* causes endocarditis (Kandi, 2016).

Antibiotics allow the organisms to eliminate from the body by inhibiting the growth of bacteria, by inhibiting the protein synthesis, acting on DNA or RNA and denature them. Antibiotics also have the ability to enter in the cell wall of bacteria where they bind with the ribosomes and stop the synthesis of protein. In the mid of 20th century antibiotics were known as "wonder drug". The concept of antibiotic was first given by Alexander Fleming when he discovered penicillin. 1950s to 1970s periods were known as the golden periods for the discovery of antibiotics. Millions of antibiotics have been produced during last 60 years. Due to the large production of antibiotics the irresponsible use of antibiotics become also increased which contributed to the discovery of resistant bacterial *sp*.

Sufficient amount of antibiotic should be taken so that it can effectively attack on target and the antibiotic which has to be taken should be activated to perform its function. To understand the antibiotic resistance mechanism five different modes of antibiotic activity have been introduced. Antibiotics kill their target bacteria by interfere with synthesis of cell wall, by inhibit synthesis of protein, stop the synthesis of nucleic acids, disturb the metabolic pathways and by disorganizing the membrane of cell. Antibiotic resistance mechanism generates by two kinds aspects either by biochemical aspect or by genetic aspects. In biochemical aspects resistance occur due to the inactivation of antibiotics (by hydrolysis, transferring of any group and redox process) modification of target by alteration in peptidoglycan structure, interference in protein or nucleic acid synthesis, changing in membrane permeability and bypassing of target. In genetic aspects resistance may occurs due to mutation which may be spontaneous or adaptive and horizontal gene transfer (resistant gene transfer to the host cell by process of recombination) (Ikeda, et al., 2015).

Antimicrobial susceptibility test for *B. cereus* was done by broth micro dilution method. *B. cereus* is the pathogen which causes blood stream infections. 29 cases of *B. cereus* infection were obtained to check the susceptibility of antibiotics against this strain. After performing broth micro dilution

technique different antibiotics were used. The result shown that *B. cereus* isolates were not resistant to vancomycin, Ciprofloxacin and imipenem. 65.5% isolates were resistant to clindamycin and 10.3% were resistant to levofloxacin (Ikeda et al., 2015).

Before 1990s, the antibiotic resistance problem was not under consideration but with the passage of time this problem became very alarming. To solve this problem the antimicrobial agent actions and the mechanisms in which these agents act on the target were examined. Resistance mechanism depends upon the pathways that are inhibited by antibiotics. Antibiotic resistance is of two type's intrinsic or active resistance and acquired or active resistance. In passive resistance bacteria doesn't have target site for the specific drug therefore the drug is not effective for the patient. Acquired resistance is that in which resistance occurs by mutation occurs in bacterial genome (Toma, & Deyno, 2015). *B subtilis* is found in gut of humans and only causes disease in those patients which are immune compromised. *B subtilis* was grown on L.B broth plates after incubation of 24 hours growth was appeared. Antimicrobial test was performed. Ciprofloxacin, vancomycin, azithromycin, chloramphenicol and cefotaxine antibiotics were used. Results concluded that vancomycin was less or intermediate sensitive to *Subtilis*, Ciprofloxacin has higher sensitivity, azithromycin shown significant sensitivity and chloramphenicol shown higher sensitivity against *B. subtilis* (Das et al., 2014).

Antibiotics have different mode of actions they act on different bacterial sites and kill the bacteria. Penicillin, cephalosporins, bacitracin and vancomycin inhibit the synthesis of cell wall. Chloramphenicol, erythromycin, tetracycline's and kanamycin stop the synthesis of proteins. Polymyxin B injured the plasma membrane. Sulfanilamide and trimethoprim inhibit the metabolites synthesis. Kanamycin changes the shape of 30S portion of ribosome, tetracycline disturbs the attachment of mRNA with tRNA and chloramphenicol attaches the 50s portion of ribosome and stops peptide formation. Antibiotics resistance is the bacterial capacity to fight against the antibiotics effect and also to interfere the normal antibiotic mechanism to increase the growth of bacteria. These resistant bacteria are capable to fight against every drug, chemical or other agents that are manufactured to treat the infections. Resistance of gram negative bacteria is increasing day by day as compared to the gram positive bacteria. A report was presented which explained that bacteria which were isolated from different samples collected from different hospitals of Pakistan were gradually resistant. *A. baumannii* species are resistant to many antibiotics at higher level. Level of multidrug resistance in Pakistan is gradually increasing.

Due to the problem of drug resistance the sensitivity and resistance of *B. subtilis, B. cereus* and *B. licheniformis* will be observed by performing antisusceptibilty test. Samples will be collected from different hospitals of Lahore. Bacteria will be isolated and ten antibiotics will be used to check the susceptibility of these bacterial *sp*. Zone of inhibition will be measured and results will be prepare.

MATERIALS AND METHODS

2.1 Sample collection

All research was done in Microbiology Laboratory of Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan. By using sterile swabs clinical samples (dental, nasal, pus and oral) were collected from Children hospital, Gulab devi hospital, Jinnah hospital, Nawaz Sharif social security hospital and University College of Medicine and Dentistry of University of Lahore.

2.2 Sample Processing

In sterile condition samples were swabbed on nutrient agar plates. Plates were incubated at 37ºC for 24 hour. Bacterial growth was observed, and mixed bacterial growth was purified by streaking single colony on nutrient agar plates by using sterile platinum loop. Plates were incubated at 37ºC overnight. Next day purified growth was observed.

2.3 Identification of bacterial isolates:

The individual colonies of bacteria were examined for their macroscopic traits such as color, size and morphology. The microscopic morphology and arrangement of purified bacteria were examined using Gram staining and spore staining. Using a sterile microbiological loop, the inoculums were sub-cultured evenly on other selective and differential media‟s Mannitol Salt Agar (MSA), Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar Base (PEMBA) and Blood agar from pure culture by streak plate method. All the plates were incubated aerobically at 37°C for 24 hours. After incubation, plates were examined for growth. These sub-cultured plates were then used in the identification and characterization of the organisms. Different biochemical tests such as Indole test, TSI test and Catalase test Nitrate Reduction test, Litmus milk reactions and Starch, Lipid, Gelatin hydrolysis tests were done for confirmation of isolated bacterial cultures on species level according to protocols described previously.

2.4 Antimicrobial Susceptibility Testing (AST):

Antimicrobial susceptibility test was performed to check the sensitivity and resistance of the particular bacteria against ten different drugs. Zones of inhibition were measured by taking different measurements.

2.4.1 Disc diffusion method:

AST was done by disc diffusion method. Inoculum was prepared in normal saline and compared to 0.5 McFarland standards. A 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate ($BaCl._2 \bullet 2H_2O$), with 9.95 mL of 1% sulfuric acid (H_2SO_4). Muller Hinton plates were prepared and incubated for 24 hours at 37ºC. Hundred microliter inoculum was swabbed on Muller Hinton agar plates. Ten commercially prepared antibiotics vancomycin, Ciprofloxacin, clindamycin, ampicillin, amoxicillin, oxacillin, azithromycin, chloramphenicol and kanamycin were placed on Muller Hinton agar plates at equal distance. Plates were incubated for 24 hours at 37ºC. Zones of inhibition were measured in milimeter.

2.4.2 Mode of action of antibiotics:

Susceptibility test for bacterial strains was done on Muller Hinton Agar by disc diffusion method. Commercially prepared discs were used for the test. Zones of inhibition were measured. Mode of action of these antibiotics mentioned in the Table 1.

Table I: Mode of action of drugs

S.NO	Full name	Abbreviation	Mode of action
1	Vancomycin	VA	Alters the permeability of cell membrane. Selectively inhibits RNA synthesis.
2	Ampicillin	AM	Bacterial cell wall inhibitor
3	Tetracycline	TE	Protein synthesis inhibitor inhibits matrix metalloproteinase.
4	Ciprofloxacin	CN	Disrupt protein synthesis irreversibly binds with 30s subunit.
5	Azithromycin	AZM	Inhibits translation of mRNA
6	Clindamycin	DA	Bacterial protein synthesis inhibitor
7	Chloramphenicol	C	Inhibits peptidyl transferase activity of bacterial ribosome.
8	Oxacillin	OX	Bacterial cell wall synthesis inhibition
9	Amoxicillin	AX	Inhibits bacterial cell wall synthesis
10	Kanamycin	S	Inhibitor of protein synthesis

RESULTS

Nutrient agar plates were prepared to observe the growth of bacteria which were collected through different sources by sterile cotton swabs. Growth was appeared on nutrient agar by swabbing the samples on agar plates. Mix growth patterns were observed on the plates. Mix growth was purified by streaking a single colony on nutrient agar plates with platinum loop. After 24 hours incubation pure growth was obtained and observed on the plates. After staining three different types of morphologies of Bacillus sp were examined under microscope. Rod shaped, short chains small colonies or single cells indicated the presence of *Bacillus subtilis* specie. Single road or short chains and slightly curved at ends indicated *Bacillus cereus sp*. Round and irregular colony with spores formation appearance was examined which relates to *Bacillus licheniformis*. Bacillus sp except *Bacillus cereus* were identified only on Mannitol Salt agar plates because they do not give growth on Eosin methylene blue and MacConkey agar. *Bacillus subtilis* gave yellowish growth on MSA, *Bacillus licheniformis* also gave yellowish growth on MSA and *Bacillus cereus* did not give any growth. Catalase, nitrate reduction, indole and Triple Sugar Iron tests were performed to identify the three Bacillus sp *B. subtilis, B. licheniformis* and *B. cereus*. In catalase test all these three *sp* formed bubbles and indicated that they are catalase positive. They gave negative indole test and positive nitrate reduction test. In Triple Sugar Iron test they gave yellowish but and yellowish slant. *Bacillus* bacteria gave no growth on Simmons' citrate agar. Catalase tests, nitrate test, indole test and Simmons' citrate test were performed to confirm the presence of *B. subtilis, B. cereus* and *B. licheniformis.*

Table 2: Table showed the results of biochemical tests performed to determine the present of *B. subtilis, B. cereus* and *B. licheniformis*

No of Species	Species	Catalase Test	Nitrate Test	Indole Test	Simmons' citrate test
1.	*B. lichenoformis*	+ve	red color	-ve	No growth
2.	*B. subtilis*	+ve	red color	-ve	No growth
3.	*B. cereus*	+ve	red color	-ve	No growth

Clinical samples such as nasal, dental caries, oral, urine, pus and skin were collected from different hospitals of Lahore. Total number of samples collected and their percentages were given in the following Table 3.

Table 3: Percentages of samples collected through different sources

Source	No of samples	Percentage
Nasal	15	12.5%
Dental	50	41.6%
Oral	20	16.6%
Wounds	9	7.5%
Acne	13	10.8%
Urine	6	5%
Sputum	7	5.8%
Total	110	99.8%

Antibiotics which were used against *Bacillus* species have different ranges of detection. Range for sensitivity, intermediate and resistance was given below in Table 4:

Table 4: Standard ranges of antibiotics susceptibility

Antibiotics	Sensitive (mm or more)	Intermediate (mm)	Resistant (mm or less)
Kanamycin	18	14-17	1
Tetracycline	15	12-14	11
Chloramphenicol	18	13-17	12
Vancomycin	17	15-16	14
Ampicillin	29	14-16	28
Ciprofloxacin	15	13-14	12
Clindamycin	21	15-20	14
Amoxicillin	20	14-17	19
Oxacillin	13	11-12	10
Azithromycin	18	14-17	13

Table 5: Zones of action of antibiotics on *B. subtilis, B. cereus and B. licheniformis*

SR NO	S	AMP	AZM	AMX	OX	TE	CN	DA	VA	C
1	17mm	10mm	18mm	11mm	8mm	15mm	23mm	0mm	12mm	11mm
2	21mm	7mm	27mm	8mm	0mm	17mm	14mm	17mm	15mm	14mm
3	15mm	0mm	0mm	0mm	0mm	16mm	20mm	20mm	21mm	16mm
4	19mm	0mm	0mm	0mm	8mm	11mm	20mm	12mm	16mm	17mm
5	11mm	9mm	17mm	12mm	0mm	22mm	16mm	14mm	14mm	9mm
6	17mm	9mm	16mm	12mm	0mm	19mm	18mm	15mm	15mm	9mm
7	18mm	0mm	14mm	7mm	0mm	18mm	17mm	18mm	14mm	18mm
8	16mm	14mm	14mm	6mm	0mm	17mm	17mm	16mm	15mm	20mm
9	16mm	9mm	17mm	11mm	0mm	20mm	16mm	17mm	15mm	23mm
10	18mm	12mm	8mm	13mm	9mm	16mm	19mm	15mm	16mm	12mm
11	15mm	0mm	0mm	7mm	0mm	19mm	18mm	16mm	10mm	18mm
12	16mm	0mm	12mm	6mm	0mm	17mm	15mm	19mm	13mm	6mm
13	16mm	19mm	14mm	18mm	8mm	21mm	16mm	15mm	17mm	17mm
14	17mm	9mm	0mm	12mm	0mm	20mm	8mm	14mm	12mm	9mm
15	17mm	6mm	11mm	6mm	0mm	18mm	16mm	19mm	17mm	11mm
16	19mm	19mm	17mm	21mm	11mm	25mm	16mm	17mm	11mm	18mm
17	18mm	7mm	14mm	0mm	0mm	16mm	17mm	18mm	15mm	11mm
18	24mm	0mm	11mm	0mm	0mm	18mm	24mm	21mm	13mm	23mm
19	16mm	0mm	19mm	9mm	6mm	12mm	18mm	16mm	18mm	15mm
20	18mm	0mm	14mm	6mm	0mm	17mm	16mm	15mm	17mm	14mm

After AST zones of inhibition were measured. This table shows the values of zone of inhibition. These values had been compared with the standard values of zone. On the basis of these standard values the sensitivity, intermediate and resistant values were estimated. The standard sensitivity range of kanamycin is 15mm or more than 15mm while resistance range of that particular antibiotic is less than 15mm. 17mm shows that the value is more than 15mm so kanamycin is sensitive. Oxacillin resistivity range is 10mm or less than 10mm; 8mm indicates that oxacillin is resistant to particular bacteria.

Table 6: Overall percentages of antibiotic activity on clinical isolates

Antibiotics	Sensitive %	Intermediate%	Resistant%
Kanamycin	95%	0%	5%
Ampicillin	5%	5%	95%
Azithromycin	15%	45%	40%
Amoxicillin	10%	10%	80%
Oxacillin	0%	5%	95%
Tetracycline	90%	5%	5%
Ciprofloxacin	90%	5%	5%
Clindamycin	5%	75%	20%
Vancomycin	25%	30%	45%
Chloramphenicol	30%	25%	40%

Table shown the overall percentages of sensitivity and resistance of ten different drugs against *Bacillus* species. Overall sensitivity of *Bacillus* species against kanamycin is 95%. Sensitivity of tetracycline and Ciprofloxacin was 90%, while clindamycin shown the minimum sensitivity against *Bacillus* species which was only 5%.Oxacillin and ampicillin were 95% resistant to *Bacillus* species while the resistance of amoxicillin was 85%. Minimum resistance was shown by kanamycin, tetracycline and Ciprofloxacin.

Table 7: Percentages of susceptibility results for *B. licheniformis*

Antibiotics	Sensitivity%	Intermediate%	Resistance%
Kanamycin	100%	0%	0%
Ampicillin	0%	25%	75%
Azithromycin	25%	50%	25%
Amoxicillin	0%	25%	75%
Oxacillin	0%	0%	100%
Tetracycline	100%	0%	0%
Ciprofloxacin	100%	0%	0%
Clindamycin	0%	75%	25%
Vancomycin	25%	50%	25%
Chloramphenicol	25%	25%	50%

100% sensitivity was shown by three antibiotics; kanamycin, tetracycline and Ciprofloxacin while no sensitivity was given by ampicillin, amoxicillin, oxacillin and clindamycin. 100% resistance was shown by oxacillin against *B. licheniformis.* No resistance was seen in the case of kanamycin, tetracycline and Ciprofloxacin.

Table 8: Percentages of susceptibility results for *B. subtilis*

Antibiotics	Sensitivity%	Intermediate%	Resistance%
Kanamycin	100%	0%	0%
Ampicillin	0%	11%	89%
Azithromycin	22%	33%	45%
Amoxicillin	11%	0%	89%
Oxacillin	0%	0%	100%
Tetracycline	89%	11%	0%
Ciprofloxacin	89%	11%	0%
Clindamycin	11%	89%	0%
Vancomycin	45%	22%	33%
Chloramphenicol	22%	45%	33%

Maximum sensitivity was shown by kanamycin, tetracycline and Ciprofloxacin against *B. subtilis.* Minimum sensitivity was given by clindamycin. 100% resistance was shown by oxacillin and 89% resistance was given by amoxicillin. Clindamycin gave maximum intermediate value.

Table 9: Percentages of susceptibility results for *B. cereus*

Antibiotics	Sensitivity%	Intermediate%	Resistance%
Kanamycin	86%	0%	14%
Ampicillin	0%	0%	100%
Azithromycin	0%	71%	29%
Amoxicillin	15%	14%	71%
Oxacillin	0%	14%	86%
Tetracycline	86%	0%	14%
Ciprofloxacin	86%	0%	14%
Clindamycin	0%	57%	43%
Vancomycin	0%	29%	71%
Chloramphenicol	28%	29%	43%

B. cereus shown different percentages of resistance and sensitivity against ten different drugs. Maximum sensitivity was shown against kanamycin, tetracycline and Ciprofloxacin while maximum resistance was shown against by ampicillin and oxacillin. Azithromycin gave maximum intermediate value.

DISCUSSION

Current study showed that kanamycin, Ciprofloxacin and tetracycline were highly sensitive to *Bacillus species.* Ampicillin, amoxicillin and oxacillin were highly resistant to *Bacillus species.* Clinical samples were collected from different hospitals of Lahore. These samples were swabbed on nutrient agar plates and after swabbing mixed growth was observed. Culture was purified by streaking. After purification gram staining was performed to examine the shape of bacteria. Crystal violet, Gram iodine, Ethanol and Safranin were used step by step in gram staining. Rod shape bacteria were observed under microscope. By using differential media bacteria were identified, three *Bacillus sp* were found, which were *B. subtilis*, *B.cereus* and *B. licheniformis*. For further identification of *Bacillus sp*, conformatory biochemical tests were performed to confirm the presence of these particular bacteria. Nitrate reduction test based on the principle of reduction of nitrate to nitrite by the addition of sulfanilic acid reagent and alpha- naphthylamine. Red color shown the reduction of nitrate to nitrite, and the test is said to be as nitrate positive test. No color change refers to as nitrate negative. Catalase is an enzyme which is produced from microorganisms. This enzyme breaks down H_2O_2 into water and oxygen. Due the formation of oxygen bubbles are produced which indicates the presence of catalase in solution. Indole test is based on the working of an enzyme tryptophanase which converts an amino acid tryptophan into indole. Indole test was performed for identification of *Bacillus sp.*

On the basis of citrate utilization Simmons' citrate test is used to distinguish gram negative bacteria. Rod shaped, short chains small colonies or single cells indicated the presence of *Bacillus subtillis* specie. Single road or short chains and slightly curved at ends indicated Bacillus cereus sp. Round and irregular colony with spores formation appearance was examined which relates to *Bacillus licheniformis*. *Bacillus subtillis* gave yellowish growth on MSA, *Bacillus lichenoformis* also gave yellowish growth on MSA and *Bacillus cereus* did not give any growth. In catalase test all these three sp formed bubbles and indicated that they are catalase positive. They gave negative indole test and positive nitrate reduction test. *Bacillus* bacteria gave no growth on Simmons' citrate agar.

At the end AST was performed to check the susceptibility of bacterial species against ten different commercially prepared drugs. After AST zones of inhibition were measured. These values had been compared

with the standard values of zone. On the basis of these standard values the sensitivity, intermediate and resistant values were estimated. The standard sensitivity range of kanamycin is 15mm or more than 15mm while resistance range of that particular antibiotic is less than 15mm. 17mm shown that the value is more than 15mm so kanamycin is sensitive.

Overall sensitivity of *Bacillus* species against kanamycin is 95%. Sensitivity of tetracycline and Ciprofloxacin was 90%, while clindamycin shown the minimum sensitivity against *Bacillus* species which was only 5%. Oxacillin and ampicillin were 95% resistant to *Bacillus* species while the resistance of amoxicillin was 85%. Minimum resistance was shown by kanamycin, tetracycline and Ciprofloxacin. 100% sensitivity was shown by three antibiotics; kanamycin, tetracycline and Ciprofloxacin while no sensitivity were given by ampicillin, amoxicillin, oxacillin and clindamycin. 100% resistance was shown by oxacillin against *B. licheniformis.* No resistance was seen in the case of kanamycin, tetracycline and Ciprofloxacin. Maximum sensitivity was shown by kanamycin, tetracycline and Ciprofloxacin against *B. subtilis.* Minimum sensitivity was given by clindamycin. 100% resistance was shown by oxacillin and 89% resistance was given by amoxicillin. Clindamycin gave maximum intermediate value. *B. cereus* shown different percentages of resistance and sensitivity against ten different drugs. Maximum sensitivity was shown against kanamycin, tetracycline and Ciprofloxacin while maximum resistance was shown against by ampicillin and oxacillin. Azithromycin gave maximum intermediate value.

In the current study three Bacillus species were reported. All strains were sensitive to kanamycin, Ciprofloxacin and tetracycline while ampicillin, oxacillin and amoxacillin were resistant against these bacteria. Overall percentage of resistance was 46% and sensitivity was 36% while 18% was intermediate. Coonrod et al. (1971). performed antibiotic susceptibility test against *Bacillus* species. They reported six Bacillus species in their paper. *B. subtilis and B. cereus* were also identified. They concluded that all the strains were sensitive to kanamycin, Ciprofloxacin, tetracycline and chloramphenicol.

Tetracycline was used against Bacillus species. Test results shown that all the Bacillus cereus strains were sensitive to tetracycline. Chemother performed the same test for Bacillus species against four drugs which were tetracycline, doxycycline, penicillin and ciprofloxacin he concluded that all Bacillus cereus strains were sensitive to tetracycline except one which was resistant to that particular drug. 20% of the resistance was shown by *B. cereus, B. subtillis and B. licheniformis*. Adimpong reported the same test in their study their study shown that clindamycin was 100% resistant *B. licheniformis*. Current study shown that resistance of clindamycin against *B. licheniformis* was 25% while no resistance was shown by *B. subtilis* against clindamycin so there is a difference between these two results. They also used many other drugs related to the present study. The drugs were chloramphenicol, Ciprofloxacin, kanamycin, tetracycline and vancomycin. Chloramphenicol shown 63% resistance against *B. licheniformis* and no resistance was shown against *B. subtillis.* Current study shown that chloramphenicol shown 75% resistance against *B. licheniformis* and 25% resistance was shown by *B. subtillis*. In the paper of Adimpong it was elaborated that Ciprofloxacin had not shown any resistance against *B. licheniformis* and *B. subtilis,* current study also shown the same results that these two species did not show any resistance against Ciprofloxacin. There is a great difference between the past result and current study in Adimpong research it was found that both *B. licheniformis* and *B. subtilis* shown 100% resistance against kanamycin but current study revealed that 100% sensitivity was shown by both of these species against kanamycin. Same results were found in case of tetracycline no resistance was shown by tetracycline in the past research and also no

resistance was shown in this current study. Adimpong added that no resistance was shown by vancomycin against *B. licheniformis* and *B. subtilis* but current study reported that 25% resistance was shown by *B. licheniformis* and 33% resistance was shown by *B. subtilis* against vancomycin.

CONCLUSION

Bacterial infections due to resistant bacterial pathogen emerge as a serious problem worldwide. Resistance occurs when an antibiotic loses its ability to kill bacteria. Unnecessary use of antibiotic is very common now a days, it is very difficult to cure antibiotic resistance problem. Therefore the current study showed that maximum sensitivity was given by kanamycin, Ciprofloxacin and tetracycline against *B. subtilis*, *B. licheniformis* and *B. cereus* and maximum resistance was showed by ampicillin, amoxicillin and oxacillin against these species. Kanamycin, Ciprofloxacin and tetracycline were highly sensitive to *Bacillus* species. Ampicillin, amoxicillin and oxacillin were resistant to *Bacillus* species. Higher percentages of resistance showed that with the passage of time antibiotic resistance is becoming a serious problem which should be solved by proper safety measurements.

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