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Preparation, Standardization of Antibiotic Discs and Study of Resistance Pattern for First-Line Antibiotics in Isolates from Clinical Samples

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Abstract
Antibiotics have been used for treatment of many infections since decades. But all antibiotics cannot be used for all infections. So, the susceptibility of the organisms to the antibiotics needs to be checked. For this purpose, disc diffusion technique has been used. In this study, low cost antibiotic discs are prepared by impregnation of antibiotic solution onto whatman filter paper grade 3. After the preparation of the disc, they are standardized by comparing their efficacy with the commercially available discs. But the major concern is about the growing resistance of microorganisms to the currently available antibiotics. Hence an attempt has been made to study the resistance pattern of the clinical isolates in the given period using low cost antibiotic discs.

Keywords: Preparation, Standardization, First-Line Antibiotics, Clinical Samples

Introduction

Objective

To prepare and standardize low cost antibiotics discs to study the resistance pattern for first-line antibiotics in isolates from clinical samples, then to compare the efficacy of the prepared disc with that of the commercial one.

Theory

Antibiotics are compounds synthesized naturally and artificially that have an inhibitory action on other microorganisms. Penicillin was the first identified antibiotics from the fungus known as Penicillium notatum. Since then many antibiotics have been identified and tested. A good antibiotic should be effective against wide range of microbes, have less side effects, it should be highly stable and should be readily absorbed by the body tissues.
The antibiotics are classified on basis of mode of action as,

1) Cell wall inhibitors: UDP-NAM-Pentapeptide and UDP-NAG are synthesized in the cytosol. They are the precursors for the cell wall synthesis. Precursor molecules are transferred to phosphorylated undecaprenyl alcohol, which is a lipid carrier in the cytoplasmic membrane and are transported to outside surface. Transglycosylases and transpeptidases reticulate peptidoglycan units. The newly synthesized peptidoglycan strand is crosslinked to form the final molecule. Eg: Bacitracin interacts with the carrier molecule and prevents precursor transport.

2) Cell membrane inhibitors: These antimicrobial agents cause disorganization of the membrane. Polymixin B and colistin interacts with the negativity charged lipids on the cell membrane of the Gram Negative microorganisms and thereby create pores on it. Nucleic acids and cations leak out of the cell, so cell death occurs. Polyene antibiotics bind to sterols and make pores in the membrane and contents leak out. Imidazoles inhibit ergosterol synthesis.

3) Nucleic acid synthesizes inhibitors: Quinolones inhibit the DNA replication by blocking the action of DNA gyrase and DNA topoisomerase IV. Rifamycins bind to the β-subunits of

the RNA Pokymerases and prevent initiation of DNA transcription. Acyclovir inhibits viruses by being converted to a triphosphate and inhibiting thymidine kinase and DNA polymerase of Herpes Viruses.

4) Protein synthesizes inhibitors: Aminoglycoside antibiotics like streptomycin, Gentamicin binds to specific ribosomal proteins and also to the major groove in the rRNA. Tetracycline antibiotics inhibit the binding of aminoacyl-t RNA to the A-site of the ribosome. Macrolide, Ketolide antibiotics have large lactone rings, bind to the peptidyl site of the 50S subunit. They also impair peptidyl transferase and interfere with the translocation of the peptide from A to P sote of the ribosome. Chloramphenicol is a bacteriostatic drug that stops bacterial growth by inhibiting peptidyl transferase activity of the bacterial ribosome.

5) Metabolic inhibitors: Sulphonamide antibiotics block biosynthesis of tetrahydrofolate which is required for the synthesis of DNA, RNA and cell wall.

Antibiotic sensitivity test: With the introduction of a variety of antimicrobial it became necessary to perform the antimicrobial susceptibility test as a routine. For this, the antimicrobial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organism. Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still in use in laboratory.

Factors influencing Antimicrobial susceptibility testing:

- pH
- Moisture
- Effect of Thymidine or Thymine
- Effects of Vairiation in Dialent Cations
- Preparation of Muller-Hinton Agar
- standardization of the inoculums

Methods if antibiotic susceptibility testing: The methods of antimicrobial susceptibility testing are divided into types based on the principle of testing involved,

1) Diffusion: Strokes method, Kirby-Bauer method
2) Dilution: Broth dilution, Agar dilution.
3) Diffusion and dilution: E-test method.

1) Disk diffusion method: The Kirby-bauer and the stokes's method are generally used for antibiotics susceptibility testing, with Kirby-Bauer method being recommended by the CLSI guidelines. The Kirby-Bauer method makes use of antibiotics disks which are placed on the plates which are inoculated with the test organism. After incubation for the required time, the zone of inhibition is measured for the test antibiotics. Depending on the diameter of the zone of inhibition, the organism can be said as sensitive or resistant to the antibiotics.

2) Dilution method: Dilution susceptibility testing methods are used to determine the minimal concentration of the antimicrobial agent required to inhibit or kill the bacteria. This can be achieved by dilution of antimicrobial in either agar or broth media. Antomicrobials are tested in log2 serial dilutions (two folds). Minimum inhibitory concentration is the minimum amount of antimicrobial agent that is required to inhibit the growth or kill the bacteria. There are two types of dilution methods that can be done; they are broth dilution and agar dilution.

3) Diffusion and dilution method: E test also known as epsilometer test is a quantitative method for antimicrobial susceptibility testing applies both dilution of antibiotics as well as diffusion of antibiotic into the medium. A predefined stable antimicrobial gradient is present on a thin inert carrier strip. When this E strip is place onto an inoculated agar plate, immediate diffusion of the antibiotics occurs. Following incubation, a symmetrical inhibition ellipse is produced. The intersection of the inhibitory zone adge and calibrated carrier strip indicates the MIC value over a wide concentration range (>10 dilution) with inherent precision and accuracy.

4) Kirby-Bauer disc diffusion method: The purpose of this test is to determine the sensitivity or resistance of a pathogenic aerobic or facultative anaerobic bacterium to various antimicrobial compounds in order to assist a physician for selecting treatment options for his or her patients. The pathogenic organism is grown on Muller-Hinton Agar plates in the presence of the antimicrobial discs. The presence or absence of growth around the antimicrobial disc is an indirect measure of the ability of the antibiotic to inhibit the organism. When a 6mm antimicrobial disc is placed on a Muller-Hinton agar plate, immediately water is absorbed into the disc from the agar. The antimicrobial in the disc diffuses into the surrounding agar. The rate of diffusion through the agar is not as rapid as the rate of extraction of the antimicrobial out of the disc, therefore the concentration of the antimicrobial is highest closest to the disc and a logarithmic reduction in concentration occurs as the distance from the disc increases. The rate of diffusion of the antimicrobial through the agar is dependent on the diffusion and solubility properties of the drug in the Muller-Hinton Agar and the molecular weight of the antimicrobial compound. Larger molecules diffuse at a slower rate than the lower weight compounds. These factors in combination result in each antimicrobial having a unique breakpoint zone size indicating susceptibility to that antimicrobial compound. If the agar plate has been inoculate with the suspension of the pathogen to be tested prior to the placing of the disc on the agar surface, simultaneous growth of bacteria and the diffusion of the antimicrobial occurs. Growth occurs in the presence of an antimicrobial compound when the bacteria reach a critical mass and can overpower the inhibitory effects of the antimicrobial compound. The estimated time of a bacterial suspension reach critical mass is 4 to 10 hours for most commonly recovered pathogens, but is a characteristic of each species and is dependent on the media used and the incubation temperature. The size of the zone of inhibition of growth is in fluemced by the depth of the agar, since the antimicrobial diffuses in the three dimensions, thus a shallow layer if agar will produce a larger zone of inhibition than a deeper layer. The point at which critical mass is reached is demonstrated by a sharply marginated circle of bacterial growth around the disc. The concentration of antimicrobial compound at this margin is called the critical concentration and is approximately equal to the minimum inhibitory concentration obtained in broth dilution susceptibility tests. Zone size observed in a disc diffusion test has no meaning in and of itself. The interpretation of resistance and

susceptibility to antimicrobials is determined through in vivo testing of blood and urine to calculate the obtainable level of a given antimicrobial that results in interpretive standards. The current interpretation standards can be found in the Clinical Laboratory Standards Institute Performance Standards for Antimicrobial Disc Susceptibility Test: Approved Standard 9th Edition.

Antimicrobial resistance: Antimicrobial resistance is the resistance of a microorganism to an antimicrobial medicine to which it was previously sensitive. Resistant organisms are able to withstand attack by antimicrobial medicines, so the standard treatments become ineffective and infection persists even after treatment. Antimicrobial resistance is the cause of the use, particularly misuse, of antimicrobial medicines and it develops when the microorganism mutates or acquires a resistance gene.

Biochemical basis of antibiotic resistance

1) Drug inactivation by microbial enzymes: Among several mechanisms involved in development of antibiotic resistance, drug modification by some enzymes play a major role in rendering the drug useless. For example, β lactamase is an enzyme synthesized by many gram positive as well as Gram negative microorganisms which convert penicillin to penicilloic acid which is therapeutically inactive.

2) Modification of target site: The other mechanism of antibiotic resistance, e.g. modification of the target is best explained by Sterptomycin and Erythromycin resistance of bacteria. Modification of S12 protein of the 30s subunit of the ribosome makes the ribosome insensitive to streptomycin. Mutations affecting proteins L4 or L12 of the 50s ribosomal subunit render it resistant to erythromycin.

3) Reduction in permeability to antibiotics: In some cases, the emergence of mutants with reduced permeability of the cell membrane to antibiotics compared to that of the wild-type strain, leads to the tolerance to the antibiotics. For example Neisseria gonorrhoae, the causative organism of gonorrhea, can gain antibiotic resistance by acquiring a methylation in the gene encoding the membrane protein 'porin', thus inhibiting the transport of antibiotics penicillin and Tetracycline into the cell.

4) Exclusion of antibiotics from the cell: Among the mechanisms involved in the resistance of bacteria of Tetracycline, energy mediated efflux is a major strategy, which does not allow the drug to accumulate in sufficient concentration to exert its inhibitory effect. It is mediated by a transmembrane export protein that fuctions as an electroneutral antiport system.

5) Overproduction of target metabolite: In some cases, the molecules, which is completely antagonized by the antibiotics, is overproduced. For example sulphonamides act by competitively inhibiting the enzyme dihydropteroate synthetase which plays a crucial role in the biosynthesis of folate. In some PABA-overproducing mutants of Staphylococcus aureus, sulphonamide molecules are outnumbered by the substrate and therefore the activity of the enzymes is not inhibited even in the presence of the drug.

Materials and Methods

Chemicals used

- Antibiotics powders- Amoxycillin, Ciprofloxacin, Cephalexin, Cefotaxime, Erythromycin, Gentamicin, Amikacin, Norfloxacin, Amoxyclav
- Distilled water
- 0.5 Mcfarland standard
- Calcium chloride

Culture used

- Escherichia coli ATCC 25922
- Staphylococcus aureus ATCC 25923
- Pseudomonas auruginosa ATCC 7553

Culture media used

- Mueller-Hinton Agar, Nutrient Agar
- Peptone water
- MacConkey Agar
- Blood Agar

Miscellaneous items used: Whatman filter paper No.3, Hole puncher, Petri plates, Test tubes, Micropipette, Tips, Inoculation loops, Sterile Aliquotes, Laminar air flow chamber, Incubator, Deep freezer, Autoclave, Refrigirator, Hot air oven and etc.

Methods

- Preparation of the filter paper discs involves the punching of holes of approximately 6mm diameter in whatman filter paper and was sterilized in an autoclave.
- The next step in the study was the preparation of antibiotic stock solutions, where a known weight of the antibiotic powder is dissolved in the sterile distilled water and was stored in the refrigerator for future use. The stock solution was diluted to obtain the working solution.
- The following step was the impregnation of the discs where the antibiotic solutions are loaded on each disc using a mechanical pipette.
- The discs were dried in an incubator and stored in small ampoules with a desiccant at minus 20 °C.

1) Preparation of filter paper discs

- For the purpose of productin of antibiotics discs, whatman filter paper No.3 was used.
- To facilitate the identification of the discs, code names and the concentration of the drug in each disc were printed on the filter paper.
- Using an ordinary office hole punching machine, holes of approximately 6mm diameter were punched. Precautions were taken to avoid overlapping of holes.
- Since the paper discs have a tendency to curl after punching, they were straightened by keeping them onto a flat surface and applying pressure on them.
- The discs were then autoclaved at 15lbs pressure for 30 minutes.

2) Preparation of antibiotic stock solution

- Standard antibiotic powders were obtained commercially.
- Known weight of antibiotic powder was dissolved in sterile distilled water to obtain the stock solution.
- The stock solution was diluted at the time of disc

preparation to obtain the working solution.
- A paper disc of 6mm diameter can absorb 0.02ml or 20µl of solutions. The concentrations of antibiotic solutions were expressed in µg/µl.

$C_1V_1 = C_2V_2$
Where C_1 = Concentration of stock solution, V_1=Volume of stock solution, C_2=Concentration of working solution and V_2=volume of working solution.
For preparation of 10ml working solution of concentration 30µg/20µl from a stock solution of concentration 20µg/µ,
20µg/µl *V_1 = 30µl/20µl *10,000µl, Therefore V_1=750µl.
So, 750µl of stock solution must be used to prepare 10ml of the working solution of the required concentration.

3) Impregnation of the discs
- Sterile discs were placed in petri dishes approximately 5mm apart.
- Using a mechanical pipette, a fixed volume of 20µl was loaded on each disc one by one, taking precautions that the tip was in slight contact with the disc.

4) Drying and Storage
- Without covering the petri dishes, the discs were allowed to dry in a clean incubator at 37°C for 4 hours.
- After drying, 30-35 discs were placed in small sterile air-tight labeled containers with $CaCl_2$ (desiccant) at the bottom.
- The discs were stored in a freezer at -20°C.
- The discs were removed from the freezer 1 to 2 hours prior to usage so that the amount of condensation that may occur when warm room air reaches the cold containers.

5) Standardization of antibiotic disc:
- The prepared antibiotics were tested for their efficacy using the Kirby-Bauer disc diffusion method and were checked if the diameter of the zone of inhibition was between the ranges for sensitivity of the organisms.
- The test organisms used were Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 7553 standard strains.
- The culture used were sub cultured a day before the sensitivity testing as 24 hours young cultures are required for accurate results.
- The inoculums were prepared from the cultures and were matched for turbidity with 0.5 Mcfarland solutions.
- The prepared antibiotic discs were placed on the inoculated agar plate along with the commercially available discs for comparison of the efficacy of the prepared discs.
- The plates were then incubated at 37 °C overnight.
- After incubation, the zone of inhibition was measured for each of the antibiotic disc and was seen if they were within the sensitivity range of the organism.

6) Collection of clinical samples:
- Clinical samples like pus, urine, blood, sputum, etc were collected from symptomatic patients attending Government Hospital, Saidapet.
- Samples were processed according to the Standard Operating Procedures. The pure isolates obtained were Klebsiella, E.coli, Pseudomonas sp., Proteus mirabilis, Proteus vulgaris, Staphylococcus aureus were subcultured in a Nutrient Agar.

7) Resistance pattern studies
- Fresh isolates were used fir sensitivity analysis.
- The inoculums of all the isolates were prepared and matched with 0.5 Macfarland solutions.
- The inoculums were then plated using pour plate method and the standardized in-house antibiotic discs were placed on the plate. The plates were then incubated overnight.
- The zone of inhibition for each antibiotic was noted and the values were compared with the standard sensitivity values.
- Depending on the values, the organism was classified as sensitive or resistant.
- After the analysis of resistance to the first-line antibiotics, the resistant organisms were further subjected to analysis by second-line antibiotics and their sensitivity patterns were also analyzed.

Results and Discussion
The efficacy of the prepared antibiotic discs was studied by comparing them with the commercially obtained antibiotic discs using the standard strains of different of bacteria. They were compared for their zone of inhibition values and the following results were obtained:

Organisms	E.coli		S.aureus		P. Aeruginosa	
Antibiotic	P (in mm)	C (in mm)	P (in mm)	C (in mm)	P (in mm)	C (in mm)
Amoxicillin	23	21	22	22	-	-
Amoxiclav	26	26	25	23	-	-
Ciprofloxacin	28	29	25	27	24	28
Erythromycin	-	-	25	29	-	-
Gentamicin	26	28	25	29	22	26
Cephalexin	25	26	29	30	-	-
Cefotaxime	28	27	29	27	28	27
Norfloxacin	24	26	28	30	-	-
Amikacin	26	25	27	24	24	26

Where, P-In-house antibiotic disc, C-Commercial antibiotic disc.

Resistance Pattern Studies

(1) Zone of inhibition values for first-line antibiotics (all values in mm)

S.No	Antibiotic / Organism	CIP	GEN	AMX	CN	CM	COT	E	S/R
1	Klebsiella	24 (R)	18(R)	6(R)	13(R)	28(R)	31(S)	-	S
2	Klebsiella	30(S)	26(S)	6(R)	19(S)	30(S)	29(S)	-	S
3	E.coli	24(R)	18(R)	16(R)	13(R)	27(R)	31(S)	-	S
4	Klebsiella	24(R)	16(R)	6(R)	16(R)	29(S)	29(S)	-	S
5	Klebsiella	30(S)	18(R)	6(R)	17(S)	27(R)	24(S)	-	S
6	Klebsiella	22(R)	19(R)	16(R)	20(S)	33(S)	28(S)	-	S
7	E.coli	22(R)	15(R)	16(R)	13(R)	26(R)	27(S)	-	S
8	Klebsiella	30(S)	17(R)	6(R)	6(R)	28(R)	31(S)	-	S
9	E.coli	30(S)	16(R)	15(R)	17(S)	27(R)	28(S)	-	S
10	E.coli	6(R)	19(R)	6(R)	6(R)	14(R)	6(R)	-	R
11	Klebsiella	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	-	R
12	Pseudomonas	24(R)	15(R)	6(R)	14(R)	16(R)	6(R)	-	R
13	E.coli	24(R)	15(R)	15(R)	6(R)	29(S)	27(S)	-	S
14	S.aureus	15(R)	6(R)	10(R)	14(R)	18(R)	13(R)	13(R)	R
15	E.coli	22(R)	15(R)	17(R)	10(R)	26(R)	25(S)	-	S
16	P. mirabilis	15(R)	16(R)	24(S)	15(R)	31(S)	13(R)	-	S
17	E.coli	20(R)	15(R)	6(R)	6(R)	29(S)	25(S)	-	S
18	E.coli	6(R)	6(R)	6(R)	10(R)	30(S)	6(R)	-	S
19	E.coli	6(R)	16(R)	10(R)	10(R)	24(R)	6(R)	-	R
20	Klebsiella	31(S)	23(R)	27(S)	23(S)	25(R)	25(S)	-	S
21	S.aureus	6(R)	6(R)	14(R)	17(R)	19(R)	17(R)	14(R)	R
22	Klebsiella	12(R)	13(R)	6(R)	10(R)	37(S)	6(R)	-	S
23	Klebsiella	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	-	R
24	Klebsiella	23(R)	24(S)	27(S)	18(S)	23(R)	6(R)	-	S
25	E.coli	6(R)	6(R)	13(R)	12(R)	6(R)	6(R)	-	R
26	S.aureus	21(R)	21(S)	6(R)	17(R)	20(R)	6(R)	23(S)	S
27	Klebsiella	20(R)	6(R)	6(R)	6(R)	6(R)	6(R)	-	R
28	P.vulgaris	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	-	R
29	S.aureus	18(R)	6(R)	12(R)	26(R)	26(R)	6(R)	22(S)	S
30	S.aureus	17(R)	6(R)	6(R)	16(R)	16(R)	14(R)	19(R)	R
31	E.coli	23(R)	12(R)	18(R)	20(S)	20(S)	25(S)	-	S
32	E.coli	26(R)	11(R)	17(R)	19(S)	19(S)	25(S)	-	S
33	Klebsiella	13(R)	18(R)	6(R)	6(R)	6(R)	32(S)	-	S
34	Klebsiella	12(R)	22(S)	15(R)	10(R)	10(R)	30(S)	-	S
35	Pseudomonas	26(R)	25(S)	6(R)	6(R)	6(R)	6(R)	-	S
36	P.mirabilis	26(R)	18(R)	23(S)	16(R)	33(S)	16(R)	-	S
37	P.mirabilis	26(R)	24(S)	24(S)	18(S)	30(S)	12(R)	-	S
38	P.vulgaris	11(R)	25(S)	13(R)	13(R)	34(S)	6(R)	-	S
39	E.coli	6(R)	24(S)	6(R)	6(R)	6(R)	6(R)	-	S
40	S.aureus	15(R)	13(R)	16(R)	10(R)	18(R)	13(R)	6(R)	R
41	E.coli	26(R)	6(R)	15(R)	14(R)	29(S)	26(S)	-	S
42	Pseudomonas	24(R)	6(R)	6(R)	6(R)	6(R)	6(R)	-	R
43	S.aureus	6(R)	15(R)	11(R)	13(R)	19(R)	17(R)	13(R)	R
44	E.coli	22(R)	15(R)	17(R)	14(R)	26(R)	26(S)	-	S

S-Sensitive
R-Resistant.

(2) Zone of inhibition values for second-line antibiotics for the isolates which were resistant to first line drugs (all values in mm)

S.no	Antibiotics / Organism	AK	ACV	CFZ	OF	CFM	AZ	S/R
1	E.coli	28(S)	6(R)	11(R)	14(R)	6(R)	22(S)	S
2	Klebsiella	6(R)	10(R)	11(R)	6(R)	6(R)	16(R)	R
3	Pseudomonas	31(S)	6(R)	29(S)	26(S)	6(R)	25(S)	S
4	S. aurens	15(R)	14(R)	15(R)	14(R)	10(R)	16(R)	R
5	E.coli	24(S)	6(R)	15(R)	6(R)	20(R)	25(R)	S
6	S. aurens	25(S)	22(R)	16(R)	19(R)	14(R)	26(S)	S
7	Klebsiella	27(S)	6(R)	27(S)	28(S)	22(R)	20(S)	S
8	E.coli	26(S)	19(R)	12(R)	17(R)	12(R)	26(S)	S
9	Klebsiella	26(S)	10(R)	14(R)	25(R)	14(R)	20(S)	S
10	P. vulgaris	27(S)	22(R)	14(R)	17(R)	6(R)	27(S)	S
11	S. aurens	6(R)	6(R)	6(R)	11(R)	12(R)	6(R)	R

12	E.coli	27(S)	22(R)	14(R)	17(R)	6(R)	27(S)	S
13	S. aurens	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	R
14	Pseudomonas	31(S)	6(R)	28(S)	27(R)	6(R)	27(S)	S
15	S. aurens	6(R)	6(R)	6(R)	6(R)	6(R)	11(R)	S

S-Sensitive
R-Resistant.

(3) Zone of inhibition values for third-line antibiotics for the isolates which were resistant to second-line drugs (all values in mm)

S.no	Antibiotics / Organism	IPM	SPF	CFN	AZM	VAN	NET	S/R
1	S.aureus	-	13(S)	20(S)	-	18(S)	26(S)	S
2	Klebsiella	34(S)	6(R)	10(R)	10(R)	-	-	S
3	Klebsiella	40(S)	12(R)	21(R)	6(R)	-	-	S
4	S.aureus	-	16(R)	25(S)	-	19(S)	21(R)	S

S-Sensitive
R-Resistant.

(4) Standard chart values for zone of inhibition (all values in mm)

Organism / Antibiotics	E.coli	S.aureus	Pseudomonas	Klebsiella	Proteus sp.
CIP	30-40	27-35	28-35	30-40	30-40
GEN	21-28	24-32	19-24	21-28	21-28
AMX	19-25	28-36	-	19-25	19-25
CN	17-22	29-37	-	17-22	17-22
CM	29-35	25-31	18-22	29-35	29-35
COT	23-29	24-32	-	23-29	23-29
E	-	22-30	-	-	-
AK	16-23	18-24	15-23	16-23	16-23
ACV	19-25	28-36	-	19-25	19-25
CFZ	25-32	19-28	23-30	25-32	25-32
OF	29-33	24-28	17-21	29-33	29-33
CFM	24-32	-	-	24-32	24-32
AZ	-	24-30	-	-	-
IPM	26-32	-	20-28	26-32	26-32
SPF	25-31	19-25	-	25-31	25-31
CFN	22-28	17-23	-	22-28	22-28
AZM	-	21-26	-	-	-
VAN	-	17-21	-	-	-
NET	22-30	22-31	17-23	22-30	22-30

Key Points: CIP - Ciprofloxacin, GEN - Gemtamicin, AMX - Amoxycillin, CN - Cephalexin,
CM -Cefotaxime, COT -Co-trimoxizole, E -Erythromycin, AK -Amikacin, ACV-Amoxyclav, CFZ -Cefixime, AZ - Azithromycin, IPM -Imipinem, SPF –Sparfloxacin, CFN – Ceftriaxone, AZM -Aztreonam and VAN –Vancomycin.

An overview of results for first-line antibiotic resistance

Isolates were obtained from clinical samples (Urine, pus, sputum, blood). A total of 44 isolates were collected. The isolates were *Klebsiella sp.* (14), *Escherichia coli* (15), *Staphylococcus aureus* (7), *Pseudomonas sp.* (3), *Proteus vulgaris* (2) and *Proteus mirabilis* (3).

They were subcultured a day before the resistance studies. The disc diffusion assay was done for all the isolates on Mueller-Hinton Agar plates using the prepared antibiotic discs. Of the isolates, 21 percent of the klebsiella were found to be resistant to all of the first-line antibiotics. 26 percent of 71 percent of the Staphylococcus sp. were found to be resistant to first line antibiotics. One-third of the pseudomonas sp. was found to be resistant to all of the first-line antibiotics. One of the two Proteus vulgaris was found to be resistant to all first-line antibiotics.

First-line antibiotics sensitivity pattern: Of the first-line antibiotics, Amoxycillin and Ciprofloxacin showed the highest resistance rates of 88 percent. Whereas, Cephalexin had a resistance rate of about 80 percent. Cefotaxime had a resistance rate of approximately 70 percent. Co-trimoxizole had a resistance rate of about 50 percent. Around 80 percent of the organisms were found to be resistant to Gentamicin. Erythromycin was used only for Staphylococcus aureus and was found to exhibit a resistance rate of 85 percent.

Amoxycillin resistance pattern: Approximately 55 percent of the Gram-negative organisms were found to be resistant to Amoxicillin. Also, 100 percent of the Gram-positive organisms were resistant to these drugs.

Ciprofloxacin resistance pattern: Nearly 85 percent of the Gram-negative species were resistant to the antibiotic. All of the Gram-positive organisms which were tested were found to be resistant to Ciprofloxacin.

Gentamicin resistance pattern: Nearly 80 percent of the Gram-negative organisms which were tested for sensitivity were resistant to Gentamicin. Also, 85 percent of the Gram-positive organisms were resistant to the drug.

Cephalexin resistance pattern: Almost 75 percent of the Gram-negative isolates were resistant to Cephalexin. Also, 100 percent of the Gram-positive isolates were resistant to the antibiotics.

Cefotaxime resistant pattern: More than 60 percent of the Gram-negative isolates were found to be resistant to Cefotaxime. And 100 percent of the Gram-positive isolates were resistant to the antibiotics.

Co-trimoxazole resistance patter: Nearly 50 percent of the Gram-negative isolates were found to be resistant to Co-trimoxazole. Whereas, 100 percent of the Gram-positive isolates were resistant to the same antibiotic.

Second-line antibiotics sensitivity pattern: After a rigorous first-line antibiotic testing, 15 organisms were found to be resistant to all of the tested antibiotics. The organisms that were resistant to first-line antibiotics include – *Klebsiella sp.* (3), *E.coli* (4), *Staphylococcus aureus* (5), *Pseudomonas sp.* (2), *and Proteus vulgaris* (1). These organisms were further subjected to analysis by second-line drugs – Amikacin, Amoxyclav, Ceftazidime, Ofloxacin, Cefixime, Azithromycin. 60 percent of the Klebsiella sp and 40 percent of the Staphylococcus aureus were found to be resistant to all of the tested second-line antibiotics. All the other tested organisms were sensitive to at least one of the second-line drugs used. Of the second-line antibiotic tested, Amikacin and Azithromycin had the least resistance of 20 percentages. Whereas, Amoxyclav and Cefixime had the highest resistance rates at 93 percent each. Ofloxacin had a resistance rate of about 85 percent and for Ceftazidime it was 73 percent.

Amikacin resistance pattern: Only 10 percent of the Gram-negative isolates were found to be resistant to Amikacin. Around 40 percent of the Gram-positive isolates were resistant to the drug.

Amoxyclav resistance pattern: 100 percent of the tested Gram-negative isolates were resistant to Amoxyclav, and around 80 percent of the Gram-positive isolates were found to be resistant to the antibiotic.

Azithromycin resistance pattern: Only 10 percent of the Gram-negative isolates were resistant to Azithromycin. Whereas, a moderate 40 percent of the Gram-positive iso; ates were resistant to the drug.

Cefixime resistance pattern: All of the analyzed Gram-negative isolates were found to be resistant to Cefixime. Whereas 80 percent of the Gram-positive isolated were resistant to antibiotics.

Ceftazidime resistance pattern: Almost 70 percent of the tested Gram-negative isolates were resistant to Ceftazidime. And 80 percent of the Gram-positive isolated were found to be drug resistant.

Ofloxacin resistance pattern: Nearly 90 percent of the tested Gram-negative isolates were resistant to ofloxacin. The Gram-positive isolated had a resistance of 80 percent for the antibiotic.

Third-line antibiotics sensitivity pattern: The four organisms which were resistant to all the second-line antibiotics were tested for sensitivity to third-line antibiotics- Imipenem, Sparfloxacin, Ceftriaxone, Aztreonam, Vancomycin, Natamycin. The organisms which were tested here included- *Klebsiella sp.* (2), *Staphylococcus sp.* (2).

Imipenem resistance pattern: All the Gram-negative isolates tested for sensitivity were found to be sensitive to imipenem. Therefore, none of the tested isolates were resistant to the drug.

Sparfloxacin resistance pattern: 100 percent of the Gram-negative isolates were resistant to Sparfloxacin and 100 percent of the Gram-postive isolates were found to be resistant to the antibiotics.

Ceftriaxone resistance pattern: 100 percent of the Gram-negative isolates were found to be resistant to Ceftriaxone, whereas none of the Gram-positive isolates were resistant to the antibiotics.

Aztreonam resistance pattern: 100 percent of the tested Gram-negative isolates were resistant to Aztreonam.

Vancomycin resistance pattern: None of the tested Gram-positive isolates were found to be resistant to Vancomycin.

Netilmicin resistance pattern: 50 percent of the Gram-positive isolates which were tested for sensitivity were found to be resistant to Netilmicin.

Conclusion

Antibiotic sensitivity testing is an important part in the treatment of infections. The most commonly used method is the Kirby-Bauer disc diffusion method. Commercially available antibiotic discs are expensive and therefore not feasible for use in laboratories in developing countries. Therefore a low cost alternative will be the preparation of antibiotic discs locally. In this study, low cost antibiotics have been prepared and also standardized by comparing their efficacy with the commercial discs. They were found to be more effective when compared with commercial discs.

Resistance to antibiotics is a major concern worldwide. Antibiotic resistance has caused a lot of morbidity and mortality all over the world. Before using an antibiotic for treatment, it is always advisable to perform a sensitivity test to analyze the resistance of the organism to the antibiotics used for the treatment. This minimizes the unwanted, reckless use of antibiotics.

In the present study, clinical isolates were used for the analysis of resistance pattern of antibiotics. The obtained isolates were then screened using disc diffusion method and the resistant organism was identified. The first screening was done using first line antibiotics. Out of 44 isolates screened, 15 were found to be resistant to all the first line antibiotics. This gives us an alarming high resistance rate of 35% for the first line antibiotics. This indicates that for nearly 1/3rd of the infections, first line antibiotics cannot be used for treatment.

All the above resistant organisms were tested for sensitivity to second line antibiotics. Out of the 15 isolates screened for second line drugs, 4 isolates were found to be resistant to all the tested antibiotics. This gives us a resistance rate of around 25%. Second - line drugs, on the other hand, are more

expensive when compared to first - line antibiotics. Therefore, they should be used only when the condition is so severe and cannot be treated using first - line antibiotics.

Last level of screening was done using third - line antibiotics for the 4 isolates which were found to be second - line resistant. All the tested isolates were found to be sensitive to at least one of the third - line drug.

The above study reveals that, antimicrobial resistance poses as a great danger signal. It is important to minimize the growth of resistant organisms by minimal and judicial use of antibiotics.

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