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Original Article

Screening Antimicrobial Susceptibility of Gentamicin, Vancomycin, Azithromycin,Chloramphenicol and Cefotaxime Against Selected Gram Positive and Gram Negative Bacteria

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ABSTRACT

Objective: Screening antibacterial susceptibility of gentamicin, vancomycin, azithromycin, chloramphenicol and cefotaxime against selected gram positive and gram negative bacteria.
Experimental approach: The goals of the assay are to detect possible antibiotic sensitivity against *Bacillus subtillis*, *Staphyllococcus aureus*, *Pseudomonas aeruginosa*, *Echerichia coli*. Growth pattern of bacterial strains without any compound stimulation were studied through time dependent measurement of bacterial OD at 600nm at every 6 hrs interval. Antimicrobial susceptibility was screened through determination of minimum inhibitory concentration (MIC) by broth micro dilution method against all these organisms where compounds showed differential inhibition. Followed by this zone of inhibition were evaluated with respect to MIC. **Finding:** Azithromycin showed minimum concentration as MIC against all organisms followed by vancomycin and cefotaxime. Amount of inhibition were visualised through study of zone diameter at MIC level. Inhibited zone diameter reflect the MIC level of compound and antibacterial susceptibility of selected two gram positive and two gram negative organism. **Discussion:** Current testing methods provide assessment of antibiotic activity using the categories susceptible, intermediate, or resistant. Lowest MIC of Azithromycin is supported by inhibited zone diameter. Gentamicin also showed high zone of inhibition which suggest that the compound exert significant bactericidal effect at MIC. Whereas other compounds irrespective of their MIC, they did not show significant zone of inhibition. **Conclusion:** Thus azithromycin can be selected as most susceptible antibiotic against these selected organisms followed by gentamicin in higher concentration. However, newer or emerging mechanisms of resistance require constant vigilance regarding the ability of each test method to accurately detect resistance.
Key words: Antibacterial susceptibility, broth micro dilution, agar diffusion, zone of inhibition.

1. INTRODUCTION

The spread of multiple antimicrobial-resistant pathogenic bacteria is a serious global human and animal health problem. Antimicrobial susceptibility

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assay on bacteria isolated from clinical specimens is essential if the bacteria's susceptibility to particular antimicrobial agents is uncertain. In case of chronic infection due to biofilm formation, bacteria are able to resist to higher antibiotic concentrations than bacteria in suspension[1, 2]. To control chronic infection, antibiotics are chosen on basis of conventional *in vitro* diffusion and dilution evolution methods[3.] Four species of gram positive and gram negative bacteria (*Staphylococcus aereus, Pseudomonas aeruginosa, Bacillus subtilis, and Escherichia coli*) are used in our study. It is estimated that 20% of the human population are long-term carriers of *S.* aureus which can be found as part of the normal skin flora and in anterior parts of the nasal passages[4, 5]. *S. aureus* is a successful pathogen due to a combination of nasal carriage and bacterial immuno-evasive strategies. *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS) etc. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections. *S. aureus* can survive from hours to weeks, or even months, on dry environmental surfaces, depending on strain[6]. *S. aureus* can survive on dogs,[7] cats,[8] and horses,[9] and can cause bumblefoot in chickens[10]. *P. aeruginosa* bacteria seem to adapt to the microgravity and the biofilms formed during spaceflight exhibited a column-and-canopy structure that has "not been observed on Earth"[11]. It is a Gram-negative, aerobic, coccobacillus bacterium with unipolar motility[12]. An opportunistic human pathogen, *P. aeruginosa* is also an opportunistic pathogen of plants[13]. It is also able to ferment arginine by substrate-level phosphorylation[14, 15]. *Bacillus subtilis*, also known as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium[16]. A member of the genus *Bacillus*, *B. subtilis* is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. *B. subtilis* has historically been classified as an obligate aerobe, though recent research has demonstrated that this is not strictly correct[17]. Although this species is commonly found in soil, more evidence suggests that *B. subtilis* is a normal gut commensal in humans. Soil simply serves as a reservoir, suggesting that *B. subtilis* inhabits the gut and should be considered as a normal gut commensal[18]. *B. subtilis* is only known to cause disease in severely immunocompromised patients, and can conversely be used as a probiotic in healthy individuals[19]. *B. subtilis* rarely causes food poisoning. *B. subtilis* is commonly used in laboratory studies directed at discovering the fundamental properties and characteristics of Gram-positive spore-forming bacteria[20]. *Escherichia coli* is a gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms)[21]. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination[23]. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 and preventing colonization of the intestine with pathogenic bacteria[24-26]. *E. coli* is gram-negative (bacteria which do not retain Crystal violet dye), facultative anaerobic(that makes ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation or anaerobic respiration if oxygen is absent) and non-sporulating[27]. Growth of Bacteria is the orderly increase of all the chemical constituents of the bacteria. Multiplication is the consequence of growth. Death of bacteria is the

irreversible loss of ability to reproduce. Bacteria are composed of proteins, Carbohydrates, lipids, water and trace elements. Bacteria growth in broth culture has been studied in great details and the common phases associated with their growth cycle is log phase, exponential phase, stationary phase, death phase. Azithromycin is bacteriostatic and inhibits synthesis of protein by binding reversibly to 50S ribosomal subunits of sensitive microorganisms, at or very close to the site that binds chloramphenicol. Cefotaxime is a β-lactam antibiotic. Gentamicin is a bactericidal antibiotic that works by irreversibly binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis [28]. Vancomycin acts by inhibiting proper cell wall synthesis in Gram-positive bacteria. Due to the different mechanism by which Gram-negative bacteria produce their cell walls and the various factors related to entering the outer membrane of Gram-negative organisms, vancomycin is not active against Gram-negative bacteria (except some non-gonococcal species of Neisseria). Chloramphenicol is a bacteriostatic drug that stops bacterial growth by inhibiting protein synthesis. Chloramphenicol prevents protein chain elongation by inhibiting the peptidyl transferase activity of the bacterial ribosome. These properties of antibiotics will be examined at first by finding the MIC followed by counter checking through zone of inhibition study on agar plates. Thus sensitivity of these antibiotics on the specific type of gram positive and gram negative bacteria will be assayed.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

All bacterial media were purchased from HiMedia (Mumbai, India). Several solvents used for the work were purchased from Sigma Chemical Pvt. Ltd. (India). All glassware were purchased from Borosil. Gentamycin is purchased from Abbott Healthcare Pvt. Ltd, (Madhya Pradesh, India). Azithromycin is protruded from Alembic Pharmaceuticals Ltd, (India). Chloramphenicol is bought from Helichem Laboraries Pvt. Ltd, Mumbai. Vancomycin is collected from United Biotech Pvt. Ltd, Solan. Cefotaxime is bought from Alkem Health Science, Sikim.

2.2 Bacterial culture and growth maintenance

Seed culture of gram positive (*S. aereus* & *B. Subtilis)* and gram negative (*P. aeruginosa* & *E. coli*) were collected from Centre for Cellular and Molecular Biology (CCMB), Hyderabad in glycerol stock. Organisms were brought back to their multiplying phase on agar solid media through sub-culture following that subsequent growth were maintain by sub-culture using L.B. broth (for *S. aereus*, *B. Subtilis*, *E. coli*) and tryptic soy broth (for *P. aeruginosa*). All strains were treated with Gentamycin, Azithromycin, Vancomycin, Chloramphenicol, cefotaxime antibiotics and incubated at 37˚C upto 48 hr. Growth rate were evaluated taking OD at 600 nm every 12 hr interval. Bacterial population of OD 0.6 at 600 nm were used for further experiments. All experiments were performed in triplicates.

2.3 Determination of Minimum Inhibitory Concentration

S. aereus, *P. aeruginosa*, *B. Subtilis* and *E. coli* were grown on liquid culture using L.B. broth and tryptic soy broth. 200µl of each seed culture was inoculated into 1800µl of fresh L.B. broth. Gentamycin (40-60µg), Vancomycin (0.5-5µg), Azithromycin (1-10µg), Chloramphenicol (5-50µg), Cefotaxime (2.17-21.72µg) were added to that corresponding tube and incubated for 24 hrs at 37ºC temperature. To determine the MIC bacterial population were recorded taking OD at 600 nm with respect to the control. Minimum concentration that showed inhibition of bacterial growth were taken as MIC of that compound. MIC were determined in triplicate tubes and average were taken.

2.4 Anti-bacterial Susceptibility Assay (Zone of Inhibition)

Known quantity of bacteria from 0.6 OD were grown on MH agar plate. The bacteria were swabbed uniformly across a culture plate to form uniform bacterial lawn. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of compounds was chosen as less than MIC, MIC and more than MIC so that inhibition at MIC can be compared effectively. Followed by this incubate at 37^0C for 24 hrs. Antibacterial property will be shown as formation of clear zone devoid of any bacterial colony around the paper disc. Average zone diameter will be measured that will be used for further analysis.

2.5 Statistical Analysis

Mean and Standard Deviation are performed in all experimental results in triplicate values.

3. RESULTS AND DISCUSSIONS

3.1 Bacterial growth

S. aureus, *B. subtilis*, *P. aeruginosa* and *E. coli* were grown under optimal condition and their growth rate were measured every 6 hour interval for upto 48 hours. All bacteria show exponential growth upto 12 hrs and after 24 hrs once again their growth rate increase exponentially (**Figure 1**). Observed result shows that *E.coli* has faster growth rate followed by *B. subtilis* and *P. aeruginosa*. *S. aureus* growth rate was as like *E. coli* for upto 36 hrs only. These observations suggest that *B. subtilis* and *P. aeruginosa*. Show same pattern of growth rate whereas *E. coli* and *S. aureus* has almost same pattern of growth rate for upto 36 hrs on incubation.

3.2 MIC

Minimum concentration that shows inhibition by these antibiotics was determined against all these selected bacteria (**Table 1**). Result shows that azithromycin has

Table 1: MIC of different antibiotics against *S. aureus*, *P. aeruginosa* , *B. subtilis* and *E. coli*.

	Name of antibiotics				
Organism	**Gentamycin (µg)**	**Vancomycin (µg)**	**Azithromycin (µg)**	**Chloramphenicol (µg)**	**Cefotaxime (µg)**
S. aureus	52±3.9	5±0.45	2±0.2	40±3.25	6.51±0.6
P.aeruginosa	56±3.85	4±0.5	1±0.25	50±4.6	6.51±0.9
B. subtilis	48±4	5±0.65	1±0.3	50±4.55	8.68±0.8
E.coli	48±3.8	4±0.75	2±0.25	40±4.05	6.51±0.75

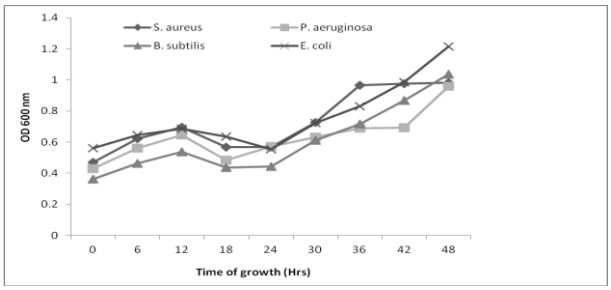

Fig 1: Growth pattern of *S. aureus, B. subtilis, P. aeruginosa* and *E. coli* under optimal growth condition shows a significant increase in bacterial number after 24 hrs of incubation.

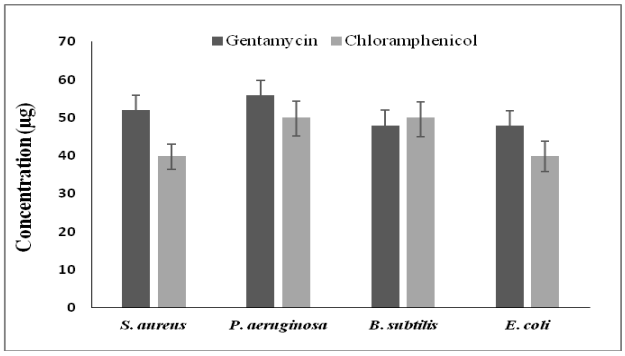

Fig 2: Minimum inhibitory concentration (µg) of gentamicin and chloramphenicol against *S. aureus, P. aeruginosa , B. subtilis* and *E. coli* which shows that chloramphenicol has less MIC than gentamicin.

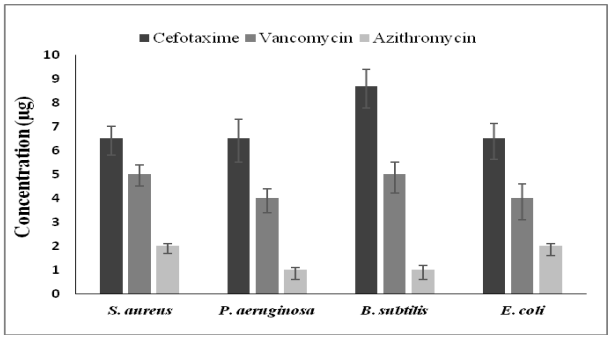

Fig 3: Minimum inhibitory concentration (µg) of cefotaxime, vancomycin and azithromycin against *S. aureus, P. aeruginosa, B. subtilis* and *E. coli* which shows that azithromycin has very much low MIC level followed by vancomycin and cefotaxime.

lowest concentration followed by vancomycin and cefotaxime against all these organisms (**Figure 2**). Gentamicin and chloramphenicol (**Figure 3**) shows MIC in higher concentration against these selected gram positive and gram negative bacteria.

3.3 Anti-bacterial Susceptibility Assay (Zone of Inhibition)

Antibacterial susceptibility of these selected antibiotics were analysed on agar solid media through measurement of zone of inhibition. All antibiotic exert concentration dependent growth pattern. Result explains that gentamicin has very high sensitivity against all organisms but maximum against *S. aureus* (**Figure 4**). Vancomycins exert less or intermediate sensitivity against all organisms. Among these the antibiotic shows highest sensitivity against both gram negative organisms followed by gram positive organisms (**Figure 5**).

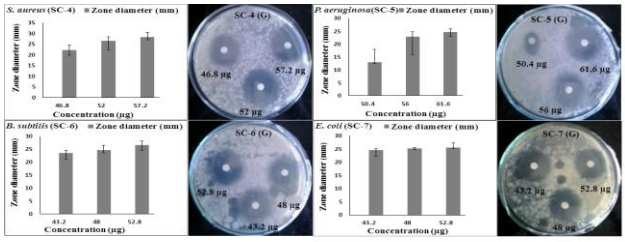

Fig 4: Sensitivity of gentamicin was assayed by agar diffusion method against *S. aureus, P.aeruginosa, B. subtilis* and *E. coli*. Gentamicin exerts concentration dependent bacterial sensitivity. At MIC the antibiotic shows highest sensitivity against *S. aureus* whereas lowest sensitivity against *P. aeruginosa*.

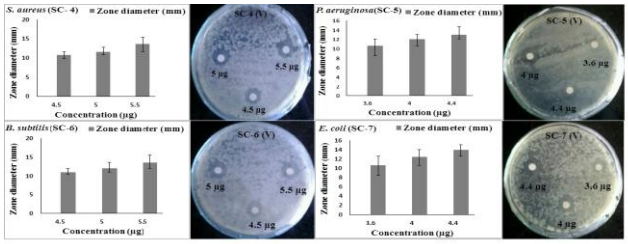

Fig 5: Sensitivity of vancomycin was assayed by agar diffusion method against *S. aureus, P.aeruginosa, B. subtilis* and *E. coli*. Vancomycin exerts concentration dependent bacterial sensitivity. At MIC the antibiotic shows similar sensitivity against all organisms.

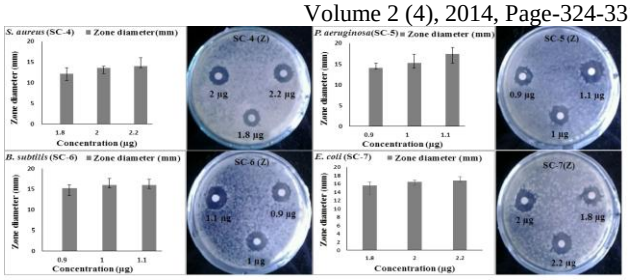

Fig 6: Sensitivity of azithromycin was assayed by agar diffusion method against *S. aureus, P. aeruginosa, B. subtilis* and *E. coli* where azithromycin shows concentration dependent bacterial sensitivity. At MIC the antibiotic shows similar sensitivity against all organisms.

Azithromycin as most susceptible antibiotic shows significant sensitivity against all organisms (**Figure 6**). Chloramphenicol showed moderate antibacterial sensitivity on all bacteria. Among these observed result chloramphenicol exert highest sensitivity on *B. subtilis* (**Figure 7**). Though cefotaxime showed very low MIC but at that concentration the antibiotic was assayed as moderate sensitive determined through zone of inhibition. Within observed results the antibiotic showed high sensitivity against gram positive bacteria than gram negative bacteria (**Figure 8**).

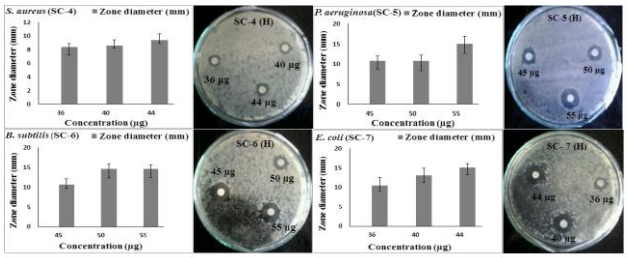

Figure 7: Sensitivity of chloramphenicol was assayed by agar diffusion method against *S. aureus, P.aeruginosa, B. subtilis* and *E. coli* where chloramphenicol shows concentration dependent bacterial sensitivity. At MIC the antibiotic shows highest sensitivity against *B. subtilis* whereas lowest sensitivity against *S. aureus*.

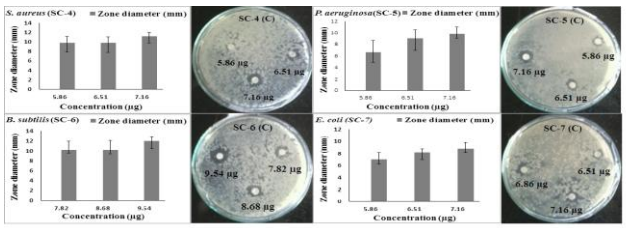

Figure 8: Sensitivity of cefotaxime was assayed by agar diffusion method against *S. aureus, P.aeruginosa, B. subtilis* and *E. coli* where cefotaxime shows concentration dependent bacterial sensitivity. At MIC the antibiotic shows more sensitivity against gram positive *S. aureus* and *B. subtilis* whereas less sensitivity against gram negative *P. aeruginosa* and *E. coli*.

4. CONCLUSION

Growth pattern is the multiplication property of bacteria through which cell division characteristics can be reflected in a systematic order. Growth curve also insight about presence of inhibitor molecules in the culture media. Here for this particular work four different bacteria were chosen. Under optimal growth condition their observed growth was gradual exponential increase pattern where *E. coli* shows maximum growth. This observation confirms organism growth behaviour under optimal condition.

Bacteria were incubated with five different antibiotics at different concentration. Bacterial growth inhibitory effect was studied and the concentration at which significant inhibition observed that were taken as minimum inhibitory concentration. This particular concentration signifies the antibacterial efficacy of that compound. Observations suggest that azithromycin has potent antibacterial sensitivity among these compounds against *B. subtilis, E. coli, P. aeruginosa* and *S. aureus*. Antibacterial susceptibility was confirmed by studying inhibitory zone diameter on agar solid media at MIC level of these compounds against *B. subtilis, E. coli, P. aeruginosa* and *S. aureus*. In this experiment gentamicin and azithromycin shows very significantly bigger zone diameter against all organisms followed by vancomycin & chloramphenicol and then cefotaxime. From these observations azithromycin can be chosen as susceptible antibacterial agent against *B. subtilis, E. coli, P. aeruginosa* and *S. aureus*. Though gentamicin shows MIC at higher concentration than vancomycin, chloramphenicol and cefotaxime but the compound exerts significant bactericidal effect as well in that concentration. Thus gentamicin can also be selected as an alternative of azithromycin against these organisms. Extensive study on azithromycin and gentamicin will be performed in future in our laboratory against these selected gram positive and gram negative organisms for better therapeutic antibacterial application.

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Conflict of interest

We declare that there is no conflict of interest.

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