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A SYSTEMATIC SHORT REPORT ON ANTIBIOTIC-RESISTANCE TRENDS AMONG BACTERIA ISOLATED FROM SEWAGE WATER

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ABSTRACT: Increasing resistance towards antibiotics among bacteria is of much concern in the clinical and scientific fields as we are nearing the verge of losing efficacy of most commonly used therapeutics that is, Antibiotics. Present work on antibiotics resistance trends is an antibiogram based systematic report involving 12 sewage water samples collected from sites near government and private hospitals. Sensitivity tests for 20 antibiotics against aerobic Gram positive and Gram negative bacteria were done using disc diffusion method. Gram positive bacteria displayed an average resistance of 50.04% (±14.26%) while all Gram negative bacterial isolates showed it to be 49.07% (±31.73%). All Gram-negative bacteria (cocci and bacilli) tested, were found to be resistant to Amoxyclav (AMC) while 75% of Gram positive cocci were also found to be resistant to it. All Gram negative cocci were found to be resistant to Cefepime (CPM) and all Gram negative bacilli were resistant to Cefoperazone (CPZ). All Gram negative bacteria (cocci and bacilli) were sensitive to Levofloxacin (LE). Chloramphenicol (CH) activity was also found to be very high against Gram negative bacteria with resistant percentage being 13%. Eight percent of all bacteria tested were found resistant to all the test antibiotics. This study reports the anti-microbial resistance pattern of bacterial isolates from sewage water samples from hospital surroundings without claiming to be the complete representation of the region under investigation as it is based on small number of samples, but it definitely indicates the severity of this global problem in regional settings.

KEYWORDS: Antibiogram, antibiotic resistance, gram negative bacteria, gram positive bacteria, sewage water.

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1. INTRODUCTION

Antibiotics may be defined as antimicrobial substances that have activity against bacteria that may kill or inhibit their growth. Some antibiotics are also known to have anti-protozoal activity, however, these are ineffective against viruses. Since their discovery, antibiotics have played central role in treatment of wide range of infectious diseases. Rigorous research has been done to identify and develop new and more effective antibiotics and as a result, we have a fair number of antibiotics with different activities, modes of action and coverage. Not only natural sources have been explored but synthetic and semi-synthetic compounds have also been developed that have anti-microbial activity. Antibiotics have application in conservative management of different infections that are common in general population as well as patients with cancer [1] like those involving skin, blood stream, lungs, hepato-biliary and intestinal tracts, urinary tracts etc. Although there are numerous antibiotics currently in use, only those involved in this study are briefly overviewed in the following paragraph using information available in DrugBank Database [2]. Ampicillin is a semi-synthetic derivative of penicillin having broad spectrum activity. There have been many forms of Penicillin that have been identified after its discovery by Sir Alexander Fleming in 1928. It is a β-Lactam antibiotic having bactericidal effect on Gram-positive and Gram-negative aerobic and anaerobic bacteria. It is used in treatment of UTI, respiratory infections, GIT infections and meningitis. Another semi-synthetic compound belonging to same class as penicillin is Cloxacillin. It is used against β-Lactamase producing *Staphylococci, Pneumococci*, group-A β-hemolytic *Streptococci*. It is a chlorinated derivative of oxacillin that inhibits cell wall synthesis. Piperacillin is another important broad spectrum, penicillin β-Lactam antibiotic used in treatment of Gram positive bacteria usually however it has reported *in vitro* activity against both Gram positive and Gram negative aerobic and anaerobic bacteria. It also inhibits cell wall synthesis [2]. Another class of antibiotics is Cephalosporins, which comprises many widely used antibiotics viz. Cefotaxime, Cefoperazone, Cefepime, Ceftizoxime. Cephalosporins disrupt the synthesis of peptidoglycan layer of the cell wall which is essential for its structural integrity. Cefotaxime is a 3rd generation cephalosporin having broad spectrum activity against Gram positive and Gram negative bacteria. It exhibits resistance to penicillinases and hence is useful in treating penicillin resistant infections. Cefoperazone is also a broad spectrum antibiotic that is semi-synthetic cephalosporin with β-Lactamase resistant tetrazoyl moiety. Ceftizoxime is an aminothiazolyl cephalosporin with extended activity spectrum against many Gram negative and hospital acquired pathogens. It is highly stable towards β-Lactamase activity and acts by inhibiting the 3rd and last stage of cell wall synthesis. Cefepime is a 4th generation cephalosporin having extended spectrum activity against both Gram positive and Gram negative bacteria. It has remarkable activity against *Enterobacteriaceae* [2]. Quinolones form a major class of antibiotics with members such as Ciprofloxacin (fluoroquinolone), Gatifloxacin (8-Methoxy-fluoroquinolone), Levofloxacin (fluoroquinolone). All these three antibiotics have a broad spectrum of activity and act by inhibiting the topoisomerase II and topoisomerase IV which are needed for bacterial DNA replication, repair, recombination and transcription [2]. A very commonly used antibiotic Erythromycin belongs to Macrolide class that inhibits bacterial protein synthesis by associating with 50S ribosomal subunits of bacteria. Its activity may be bacteriostatic or bactericidal depending upon the microbe against which it is used and its concentration. It is generally used in treatment of *Streptococci, Staphylococci, Pneumococci* and is also reported to affect Gram negative bacteria and few fungi. Co-trimoxazole exhibits its antibacterial activity against both Gram-positive and Gram-negative bacteria. Tetracycline, on the other hand, is a polyketide antibiotic having bacteriostatic effect. It is a short acting antibiotic which inhibits translation in bacteria by binding to 30S ribosomal subunit. Linezolid is a synthetic antibiotic belonging to oxazolidinones, which is used in treatment of aerobic Gram positive bacterial infections. Methicillin and vancomycin resistant *Staphylococci*, vancomycin resistant *Enterococci,* penicillin resistant *Pneumococci* and few others are also susceptible to it [2]. Gentamicin, a well-known aminoglycoside antibiotic is broad spectrum in its activity and acts by binding to the bacterial 30S subunit, leading to misreading of tRNA and thus inhibiting protein synthesis (translation). It is useful primarily in combating infections caused by aerobic Gram negative bacteria and also in some Gram positive bacterial and mycobacterial infections. Another antibiotic belonging to aminoglycoside class is Amikacin (Kanamycin derivative), which is useful in infections caused by aerobic Gram-negative bacteria, some mycobacteria and Gram positive bacteria. It also inhibits bacterial protein synthesis by binding to 30S ribosomal subunit [2]. Clindamycin is a semi-synthetic lincosamide antibiotic effective against enteric and other bacteria and acts by inhibition of protein synthesis upon binding to 50S ribosomal subunits. Vancomycin on the other hand, is a branched tricyclic glycosylated non-ribosomal peptide considered the drug of last resort. Use of Vancomycin in clinical practice began to treat Methicillin resistance in *S. aureus* and coagulase-negative *Staphylococci* in the year 1972 [3]. It is used only after treatment with other antibiotics has failed. It inhibits bacterial cell wall assembly and is also toxic for inner ear and kidneys [2]. A serious side-effect inducing drug that is less often used is Chloramphenicol. It is effective against many bacteria and is generally used for treatment of life threatening conditions like typhoid. It acts by blocking peptidyl transferase and thus inhibiting protein synthesis [2]. *Antimicrobial Resistance: A rising concern:* Application of holistic principles of hygiene and public health have increased the life expectancy to more than thirty years and improved life quality since the beginning of 20th century and antibiotics contributed to a great extent to this achievement. Since then, antibiotics are the main anti-infective agents used for treatment and prophylaxis. In addition to this, these wonder drugs have been used in agriculture [4], veterinary medicine [5, 6], dairy industry [7] and many other fields. Antibiotics brought a revolution in the field of medicine in the 20th century [8]. With passage of time, certain factors like changing demographic patterns, food distribution, socio-economic and environmental changes and emerging pathogens with antimicrobial resistance etc. increasingly began to pose serious health threats even in the developed nations [9]. Antimicrobial resistance emerged as a multifaceted phenomenon that is actually naturally occurring process when microbes are exposed to antibiotics [10]. Bacteria evolve and develop resistance against antibiotics mainly by three mechanisms-(a) Decreased uptake of the antibiotic; (b) Target modification and (c) Enzymatic inactivation of the antibiotic [11]. The problem of antimicrobial resistance development has been classified by the World Health Organization as a serious threat that is no longer a prediction for future and is a continuously ongoing process happening right now in every part of the world and has the potential to affect anybody, in any country and of any age [12]. Utilization of antimicrobials at a very large scale has brought this challenge of resistance development among microbes. The misuse and over use of antibiotics have further worsened the situation. Over dosing, intake for extended periods, use of broad spectrum antibiotics, taking antibiotics without consulting a qualified medical personnel are some of the factors that increase the burden of antimicrobial resistance. Not only in India but many other countries, antibiotics use is unregulated and are sold over the counter without prescription [13]. Incorrect prescriptions of antibiotics also contribute to increase in antibiotic resistance [14]. In hospital settings particularly, the intensive and prolonged usage of these drugs largely contributes to emergence and spread of highly antibiotic resistant nosocomial infections, however, other factors like immunosuppression among patients of AIDS, cancer, transplant recipients, weak elder patients, intense clinical therapy, invasive surgical methods etc. also aggravate it extensively [15]. There is even more complexity regarding the issue of antibiotic resistance as defining and quantifying the relationship between antibiotic use and resistance is very difficult [16]. Further, disposal and management of unused and expired antibiotics is a major concern as these also contribute to resistance development upon entering the environment [17-19]. Spreading awareness among general public about proper disposal of these pharmaceutical substances is urgently needed [19]. In addition to drug take-back programs, other economical ways that can reduce generation of unused and expired medicines have been looked at as effective approaches [20]. Further, attempts have been made to address this issue by looking for alternatives for antibiotics and testing efficacy of combinatorial therapeutic approaches [21-23], alternative therapies [24], development of new antibiotics [24, 25] etc. However, development of new antibiotics, especially against gram negative bacteria, is quite a challenge and it is known that getting new antibiotics in near future is extremely difficult [26, 27]. The success of these alternate approaches still needs to match that of antibiotics and there is a need to develop a confidence among the masses to choose these alternatives over antibiotics for treatment of different ailments. Efforts have been made in high resource settings where evidence based strategies have improved the antibiotic usage pattern but in low resource settings, current research and surveillance in relation to antimicrobial resistance are extremely limited and are largely focused on intensive care units only [28]. More efforts like antimicrobial stewardship, intense research and surveillance, development of bedside diagnostic tools for infections and antimicrobial susceptibility are required to improve antibiotics use in low resource settings [28, 29]. A fair knowledge about local microbial resistance profile and prevalence is a must for guiding antimicrobial therapy [30]. Some previous studies that have been done in this relation in different regions of India have shown alarming facts about the antimicrobial resistance patterns prevailing there. For instance, it has been reported that the Byramanagala tank in South Bangalore, India had become a cesspool of multi-drug resistant superbugs (multi-drug resistant *Salmonella, Klebsiella, Staphylococci*) that would be a major health concern there [31]. In another instance, the study showed significant concentration of antibiotics being discharged in Yamuna river waters with Ampicillin concentration being maximum [32]. It was also suggested in the same study that medicines consumed for healthcare are most often released in sewage and after the treatment of this water at sewage treatment plant, it reaches lakes and rivers to contaminate them with antibiotics and their residues [32]. Through continuous monitoring and surveillance in many parts of the world, antibiotics presence has been found in sewage treatment plants [33, 34] and rivers but monitoring of this problem is scarce in the Indian subcontinent [32]. Thus, it becomes clear that keeping an eye on the prevailing microbial flora of a defined area (such as a healthcare setting or a town), its antibiotic sensitivity and resistance patterns, half-yearly or annual trends and alterations etc. is increasingly important as it can help monitor and reduce the indiscriminate and unnecessary use of antibiotics. By doing so, the medical practitioners can prescribe more precise and effective antibiotics. The use of narrow spectrum antibiotics can be increased over broad spectrum antibiotics, which will help in specific treatment of the pathogen without adversely affecting the normal flora of the patient.

Aim and Objectives

The present study aimed at understanding the response of aerobic Gram positive and Gram negative bacteria isolated from sewage water in regions surrounding hospital settings (in Gwalior region) towards different antibiotics so that their resistance and sensitivity patterns can be determined.

2. MATERIALS AND METHODS

Twelve sewage water samples were tested for bacterial growth and the culture-sensitivity tests of the isolates were done. The standard procedure for culture with maintenance of aseptic conditions was carried out in each case. Samples were collected from areas surrounding government and private hospitals in Gwalior (M.P.), India, in sterile collection containers. Samples were centrifuged to remove solid debris and inoculated in nutrient agar plates. Incubation was done at 37° C for 24-48 hours in aerobic conditions. Separate pure colonies of different morphological properties were selected and sub-cultured. Gram staining was performed for the bacterial colonies after 48 hours of incubation and antibiograms were determined using antibiotic rings obtained from HiMedia Laboratories Pvt. Ltd. and Pathoteq Biological Laboratories (India). The overall data generated was systematically analyzed to draw out relevant information regarding antibiotic resistance trend. The details of the bacteria isolated and antibiotics used are illustrated in Table 1 and 2 respectively.

Table 1: Summary of Bacterial Isolates

Type of Bacterial Isolate	Number of Bacterial isolates
Gram Positive Cocci	13
Gram Positive Bacilli	12
Gram Negative Cocci	6
Gram Negative Bacilli	7
Total (All isolates) =	**38**

Table 2: Details of Antibiotics used for Sensitivity Determination

Name of Antibiotic (Abbreviation)	Concentration (mcg)	Used against (in this study)	Spectrum (BS/NS)#
Ampicillin (AMP)	10	Gram +ve and –ve both	BS
Co-Trimoxazole (COT)	25	Gram +ve and –ve both	BS
Tetracycline (TE)	30	Gram +ve and –ve both	BS
Cefotaxime (CF)	30	Gram +ve and –ve both	BS
Ciprofloxacin (CIP)	5	Gram +ve and –ve both	BS
Levofloxacin (LE)	5	Gram +ve and –ve both	BS
Gentamicin (GEN)	10	Gram +ve and –ve both	BS
Amoxyclav (AMC)	10	Gram +ve and –ve both	BS
Cefoperazone (CPZ)	75	Gram +ve and –ve both	BS
Cefepime (CPM)	30	Gram +ve and –ve both	BS
Amikacin (AK)	30	Gram–ve	BS
Linezolid (LZ)	30	Gram +ve	BS
Erythromycin (E)	15	Gram +ve	NS
Clindamycin (CD)	2	Gram +ve	NS
Vancomycin (VA)	30	Gram +ve	NS
Cloxacillin (COX)	1	Gram +ve	BS
Piperacillin (PC)	100	Gram–ve	BS

Chloramphenicol (CH)	30	Gram–ve	BS
Ceftizoxime (CI)	30	Gram–ve	BS
Gatifloxacin (GF)	10	Gram–ve	BS

#BS= Broad Spectrum Antibiotic; NS=Narrow Spectrum Antibiotic

3. RESULTS AND DISCUSSION

Resistance Pattern of Gram Positive Bacteria

All of the 25 Gram positive bacterial isolates did not display a great variation in their resistance pattern with an average of 50.04% (±14.26%) overall resistance. This smaller range of variation is indicative of similar responses of the Gram positive bacteria towards all antibiotics under consideration. It can be further speculated that efficacy of these antibiotics can only be improved towards these bacteria by increasing the dose (Figure 1, 2 and 3a). However, notable difference in responses was observed among their bacilli and cocci forms like Gram positive bacilli exhibited resistance towards amoxyclav (AMC, 10 mcg) in about 43% cases while Gram positive cocci displayed resistance towards it in nearly 78% cases (Figure 1 and 2). Resistance towards Linezolid (LZ, 30mcg) and Co-trimoxazole (COT, 25mcg) was also found in more than 60% Gram positive cocci while that towards Cefotaxime (CF, 30 mcg) was found in ~25% Gram positive cocci (Figure 2).

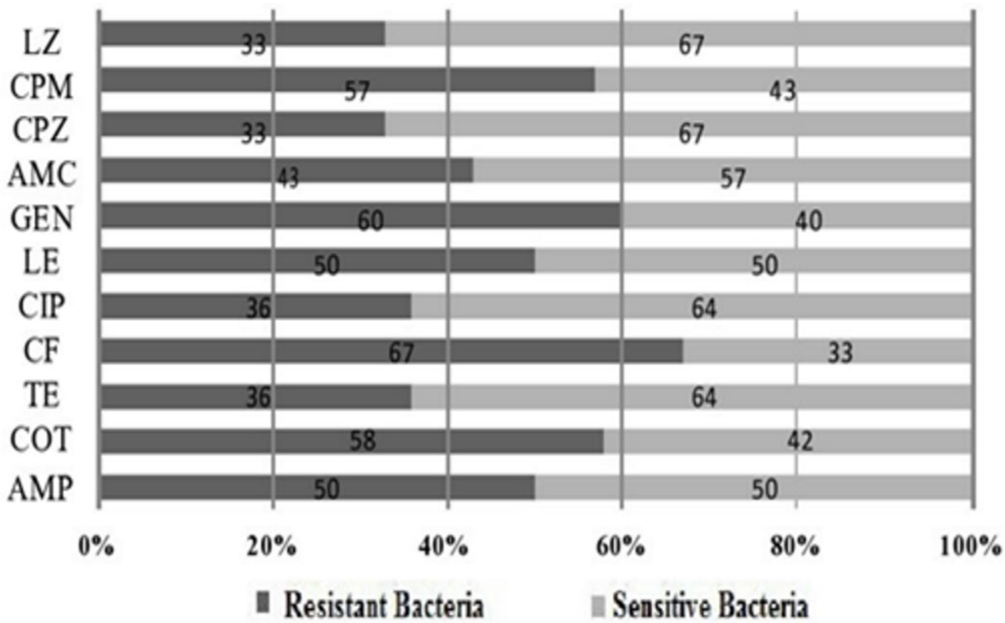

Figure 1: Bar graph representing %age of Resistant and Sensitive Gram Positive Bacilli toward different antibiotics

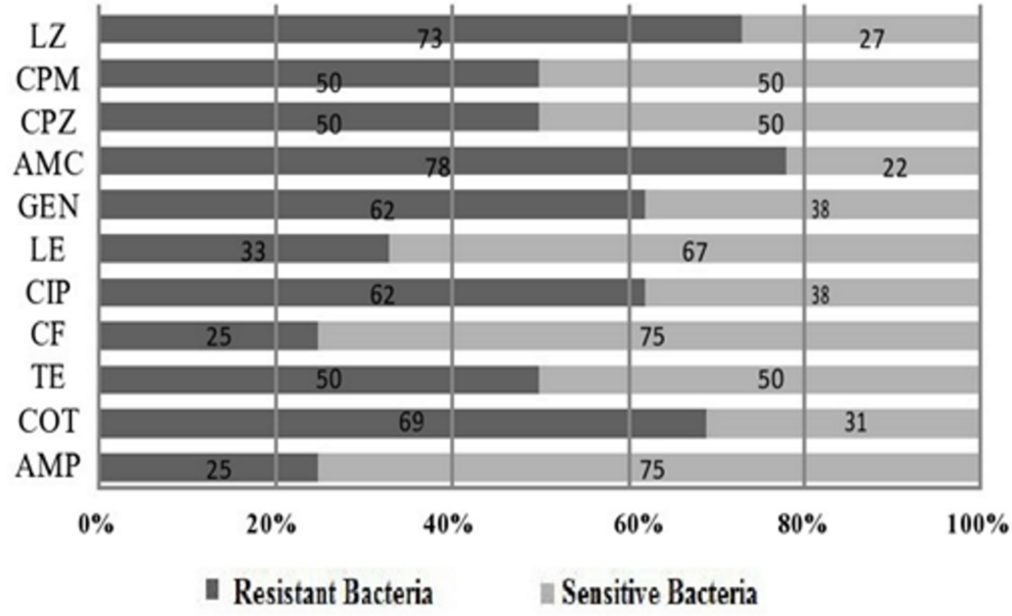

Figure 2: Bar graph representing %age of Resistant and Sensitive Gram Positive Cocci towards different antibiotics

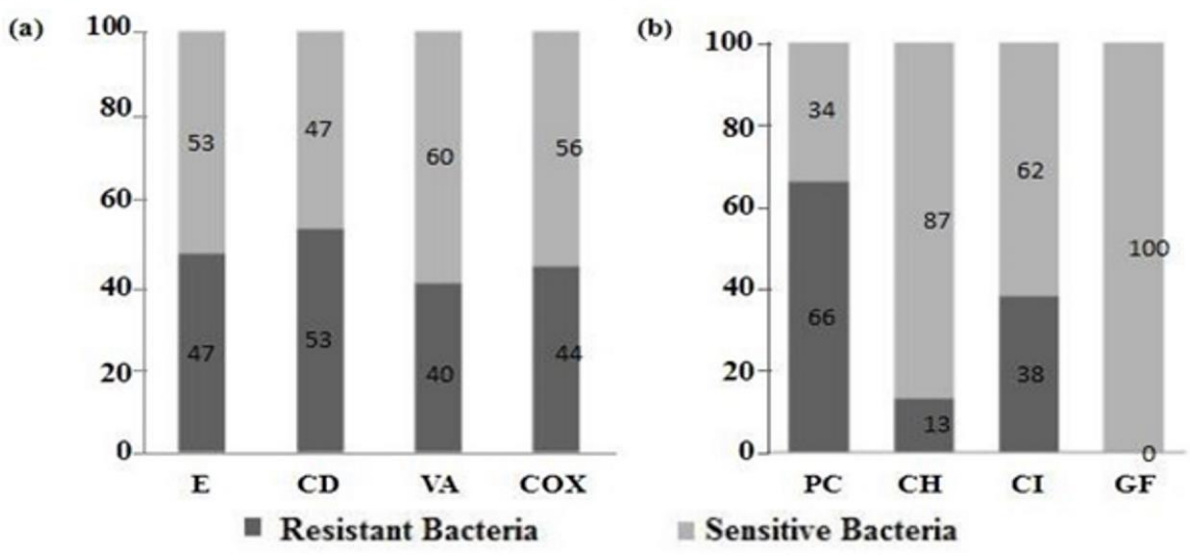

Figure 3: Bar graph representing %age of Resistant and Sensitive Gram Positive Bacteria (a) and Gram Negative bacteria (b) towards different antibiotics

Resistance Pattern of Gram Negative Bacteria

A total of 13 Gram negative bacterial isolates considered in this study exhibited an overall resistance of 49.07% (±31.73%) with more deviation due to more variability in response of the isolates towards different antibiotics. This also points towards need to switch to antibiotics for which most Gram negative bacteria are sensitive rather than increasing the dose in case of Gram negative bacterial infections. All Gram negative bacteria (cocci and bacilli) were sensitive to Gatifloxacin (GF, 10mcg) while all Gram negative cocci were sensitive to Levofloxacin (LE, 5mcg) and Cefotaxime (CF, 30mcg). The Gram negative bacilli were also found to be sensitive to Levofloxacin (LE, 5mcg).

Chloramphenicol (CH, 30mcg) activity was also found to be very high against Gram negative bacteria with resistant percentage being around 13%. This indicates that Levofloxacin, Chloramphenicol and Gatifloxacin at the mentioned concentrations can yield better results when prescribed for Gram negative bacterial infections (Figure 3b; 4a and 4b).

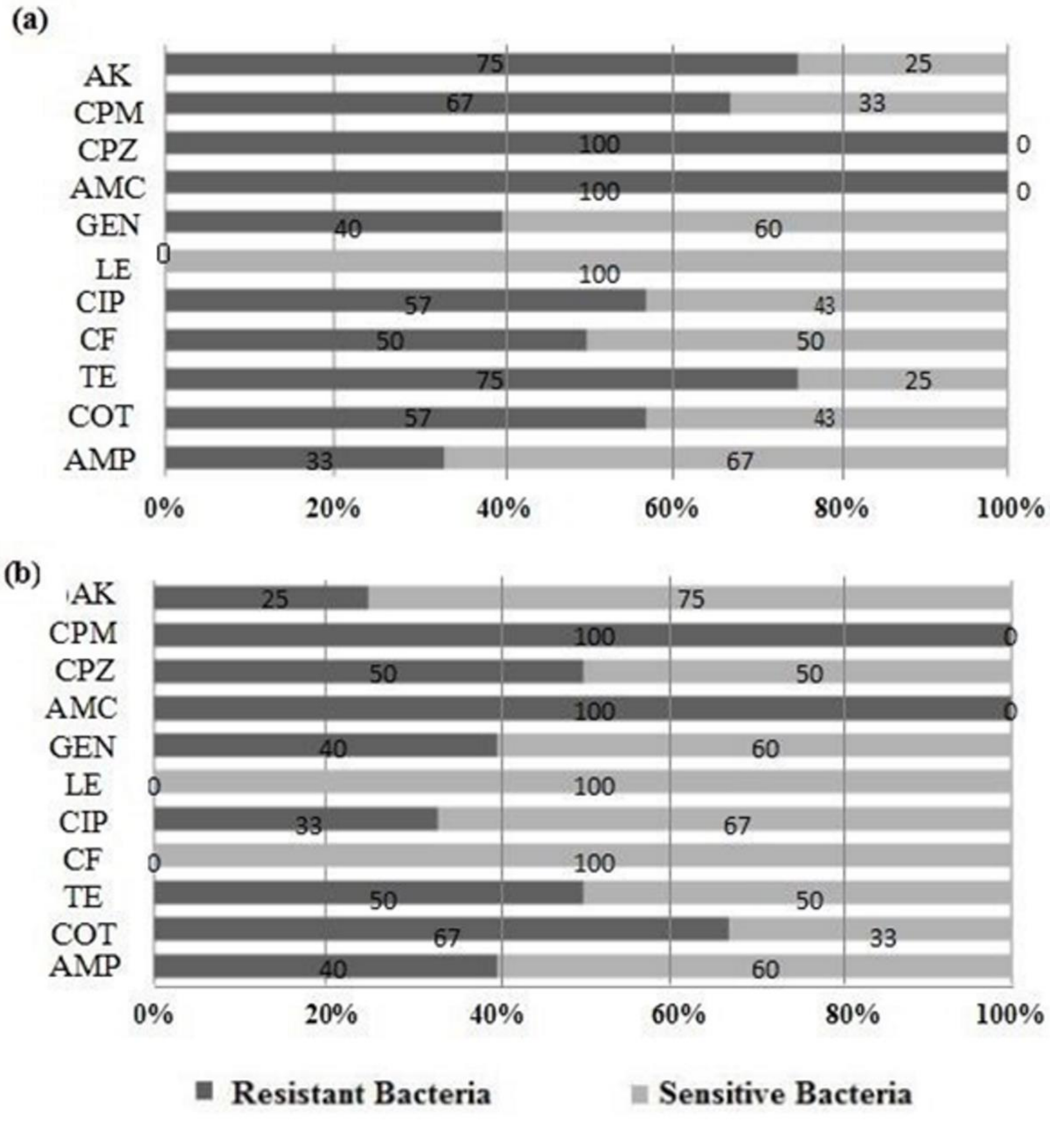

Figure 4: Bar graph representing %age of Resistant and Sensitive Gram Negative Bacteria towards different antibiotics: Gram negative bacilli (a); Gram negative cocci (b).

Degree of sensitivity exhibited by bacterial isolates

Most of the bacterial isolates that were sensitive towards the antibiotics under consideration exhibited high degree of sensitivity as their zones of inhibition sized 16-25mm and above (3+). However, the zone sizes were found to be variable among Gram positive bacteria towards two antibiotics-Levofloxacin (LE) and Cefepime (CPM) and among Gram negative bacteria towards Gentamycin (GEN) (Table-3).

Table 3: Modes of Observed Sensitivity

Antibiotic	Gram Positive Bacteria	Gram Negative Bacteria
AMP	3+	3+
COT	3+	3+
TE	2+	3+
CF	3+	3+
CIP	3+	3+
LE	2+ (=3+)	1+
GEN	3+	1+ (=2+, =3+)
AMC	2+	3+
CPZ	3+	1+
CPM	3+ (=1+)	1+
LZ	3+	N/A
AK	N/A	2+
E	3+	N/A
CD	2+	N/A
VA	2+	N/A
COX	1+	N/A
PC	N/A	3+
CH	N/A	3+
CI	N/A	3+
GF	N/A	2+

a. Categories of Antibiotic Activity on the basis of size of Zone of Inhibition (mm): 07mm-09mm= 1+; 10mm-15mm= 2+; 16mm-25mm or above= 3+.

b. N/A= Not Applicable.

Significant Percentage of Highly-Drug Resistant Bacteria

Although 74% of bacterial isolates were sensitive to 4 or more antibiotics up to different degrees but a fair %age of the isolates were resistant also. Eight percent of the studied bacteria were resistant to all test antibiotics while the %ages of those sensitive to one, two and three antibiotics were: 8%, 2% and 8% respectively (Figure 5).

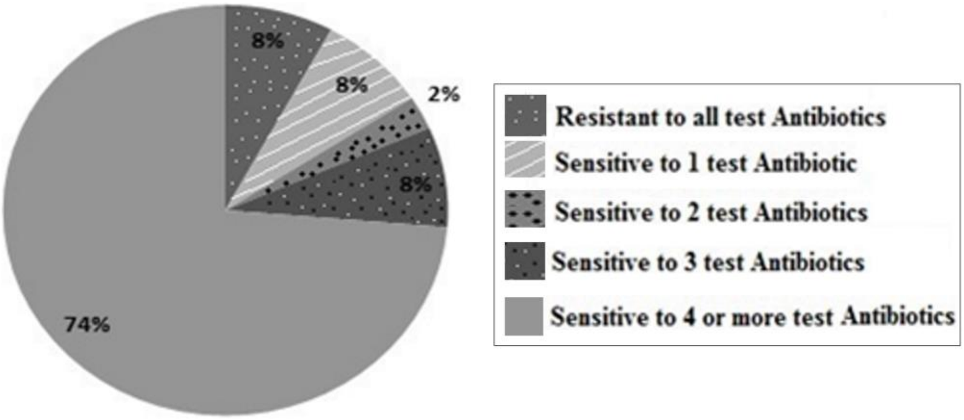

Figure 5: Percentages of Resistant and Sensitive Bacteria towards test Antibiotics

4. CONCLUSION

Antibiotics resistance is a matter of serious concern which should be tackled keeping in mind the local trends. This study highlights the prevailing resistance pattern among bacterial isolates from sewage water samples collected from areas surrounding hospitals, where possibility of isolating antibiotic resistant bacteria is fairly high. It indicates that Gram positive bacterial infections can be better handled upon increasing the dose while Gram negative bacterial infections can be better dealt by using the specific antibiotics and/or switching to more appropriate antibiotic as per the microbiological antibiogram report. Resistance towards all antibiotics in 8% test bacteria is a matter of concern.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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