Vol. 21(1), pp.16-25, January 2022
DOI: 10.5897/AJB2021.17394
Article Number: 3C7DC4268586
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Full Length Research Paper

Isolation and characterization of multidrug resistance bacteria from hospital sewage samples, Maharashtra, India

Bhaurao Bapurao Choushette[1*] and Rajendra A. Satpute[2]

[1]Plot No- 15, Near D.ed College, Pahadsingnpura, Caves Road Aurangabad, India.
[2]Government Institute of Science, Aurangabad, India.

Received 5 August, 2021; Accepted 7 January, 2022

Multiple antibiotic resistance is a major cause of clinical infections worldwide. This study determined the pattern of multidrug bacterial resistance in hospital sewage samples from the Marathwada region of India. Forty-eight isolates of bacteria were obtained from 6 locations of Aurangabad. An antibiotic sensitivity test was carried out using the disc diffusion method. Among all the antibiotics tested, the highest level of resistance was observed in the beta lactam class (85%), followed by Tetracycline (58%), Cephalosporin (58%), quinolones (52%) and gentamycin (45%). *Escherichia coli* and *Klebsiella pneumoniae* are the most prevalent bacteria, showing antibiotic resistance to all tested antibiotics with a MAR index of 1. It is concluded that hospital sewage water could be a reservoir of antibiotic resistant bacteria, which may further contaminate drinking water bodies, potentially presenting a public health risk to the general populace.

Key words: Antibiotics, *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas species, Staphylococcus aureus, Streptococcus pyogenes.*

INTRODUCTION

Antibiotics are compounds produced by micro-organisms and that are capable of inhibiting bacterial growth. Nowadays, the term antibiotic is broadly used for all those compounds that can be used against the bacterial infection. However, antibiotic resistance is increasing, and it is projected that by 2050, antibiotic resistant organisms will contribute to over 10 million deaths annually worldwide (De Kraker et al., 2016). This is mainly because of the slow pace of developing new antibiotics (De Kraker et al., 2011). The problem of antibiotic resistance has attracted the attention of World Health Organization (WHO) and several other stakeholders. The WHO announced in 2011 that antibiotic resistance is an urgent priority of research area (WHO, 2011) and several countries, including India (Government of India, 2017) accordingly framed their national health action plans for managing drug resistant bacteria (Smith et al., 2016).

*Corresponding author. E-mail: bhau64te@gmail.com. Tel: 0086-851-28927815.

The problem of antibiotic resistance occurs due to the uncontrolled and excessive use of antibiotics by hospital and home care patients (Sidhu et al., 2016). This leads to the spread of resistant genes in water systems (Mukhopadhyay et al., 2012). For instance, Sidhu et al. (2016) analyzed and demonstrated the presence of *E. coli* in the drinking water from different schools in Northern India. Many multiple drug resistant bacteria like *Pseudomonas aeruginosa*, coliforms and *Enterococcus* species have been isolated from household water samples in Karnataka, India (Mukhopadhyay et al., 2012). Mulamattathil et al. (2014) documented the multiple antibiotic resistance profile in bacteria from sewage water samples (Mafikeng, South Africa).

In rural communities of developing countries like the India, untreated water from rivers, dams, and streams is directly used for drinking (Biyela et al., 2004). These water resources could possibly be contaminated with microbes from sewage water through rainfall runoff and other sources (Obi et al., 2002). Most of the areas are semi-arid with very low rainfall and a high evaporation rate, so water should be reused (Mckenzie et al., 2003). For obtaining good quality water, there is need for proper waste water treatment procedures. Biological waste water treatment processes may selectively increase the antibiotic resistance in water, which is a major health concern issue of reuse of water (Mulamattathil et al., 2000). Release of hospital sewage directly into the water ecosystem further contributes to the antibiotic resistant bacteria. Therefore, there is pressing need to focus on this aspect locally and globally. Hence, the current study has been undertaken to evaluate the presence of potential pathogenic bacteria in hospital sewage samples from Marathwada region of Maharashtra, India, as well as to determine the antibiotic resistance profiles of the isolated bacteria.

MATERIALS AND METHODS

Collection of sewage samples

One hundred milliliters of hospital sewage samples were collected from six different sites in Aurangabad, Maharashtra region, in sterile screw cap tubes and brought to the laboratory in a cooled condition. Serial dilutions [10^{-5}] of each samples were prepared, and 1 ml of each sample were spread on the nutrient agar medium. Further plates were incubated at 37°C for 24 h and different colonies were obtained. Pure bacteria were procured by streak plate method utilizing Mueller-Hinton agar (Hi-Media, India) or Nutrient Agar [Hi-Media, India]. The isolated bacteria were characterized on the basis of colony morphology study. All the pure colonies were subjected to Grams staining and distinguished bacteria in two groups, viz: Gram positive and Gram negative. These pure cultures were further subjected to biochemical tests according to Nandi and Mandal, (2016), Holt (1984) and Forbes et al. (2007). Morphologically different colonies of bacteria were maintained on Nutrient Agar or Cystine tryptone agar (Hi-Media, India) stabs, at 4°C for further studies. Of the 30 isolates obtained, eight were further used for antibiotic sensitivity testing.

Collection of samples

A total of six hospital sewage samples were collected during the study period, from different sampling sites of Marathwada region, Maharashtra, India and labelled as S1, S2, S3, S4, S5 and S6 (Figure1) in sterile plastic bottles. After dilution and inoculation of each sample on nutrient agar plates, many colonies were obtained. Three Gram positive and 5 Gram negative bacteria were isolated from each of the 6 hospital sewage samples (Table 1). Further, their sensitivity to different classes of antibiotics namely beta lactam group, cephalosporin group, tetracycline, quinolones and aminoglycosides was analyzed. It was observed that all of the 6 samples carry antibiotic resistant bacteria for different antibiotics (Tables 2 and 3).

Antibiotic sensitivity test

Isolated bacteria were further subjected to the antibiotic sensitivity tests by disc diffusion method given by Kirby-Bauer. It was performed through following steps.

Preparation of the test organisms for sensitivity test

Three colonies from each sample were taken and subcultured in sterile nutrient broth aerobically at 37°C for 24 h. Broth cultures of the isolates were centrifuged at 3000 rpm for 10 min. The sediments were diluted with sterile phosphate buffer saline (PBS) and adjusted to the 10^8 CFU/ml using McFarland matching standard (mixture of 0.6 ml of 1% $BaCl_2.H_2O$ and 99.4 ml of 1% concentrated H_2SO_4) using spectrophotometer at 540 nm.

Antibiotic sensitivity testing

100 µl of each aliquot was spread on nutrient agar or Mueller Hinton agar medium. By using sterile forceps, antibiotic sensitivity discs were applied on the surface of the medium. The set-up was incubated aerobically at 37°C for 24 h. The inhibition zone diameters were measured using meter rule after 24 h, incubated and recorded.

The results, in terms of ZDI (zone diameter of the inhibition) values of the test antibiotics, were interpreted following the guidelines of the Clinical and Laboratory Standards (CLSI) Institute (2011). As per the CLSI guidelines, bacteria were classified into three groups viz. resistant, intermediate and sensitive to a particular antibiotic.

Determination of antibiotic resistance pattern

Bacteria showing antibiotic resistance to three or more antibiotics were considered as the multiple antibiotic resistant bacteria [MAR] and MAR index value for each sample using the following formula.

MAR index = M/n

Where, M is number of antibiotics to which the isolate showed resistance and n is the number of total antibiotics used in the test (Krumperman, 1983) that was calculated. Generally, MAR index value higher than 0.2 indicates the isolate is multiple antibiotic resistant (Adefisoye and Okoh, 2017).

To know the prevalence of antibiotic resistance as per the sample collection sites, ARI [Antibacterial resistance index] was determined using the formula described by Krumperman (1983), which is mathematically expressed as

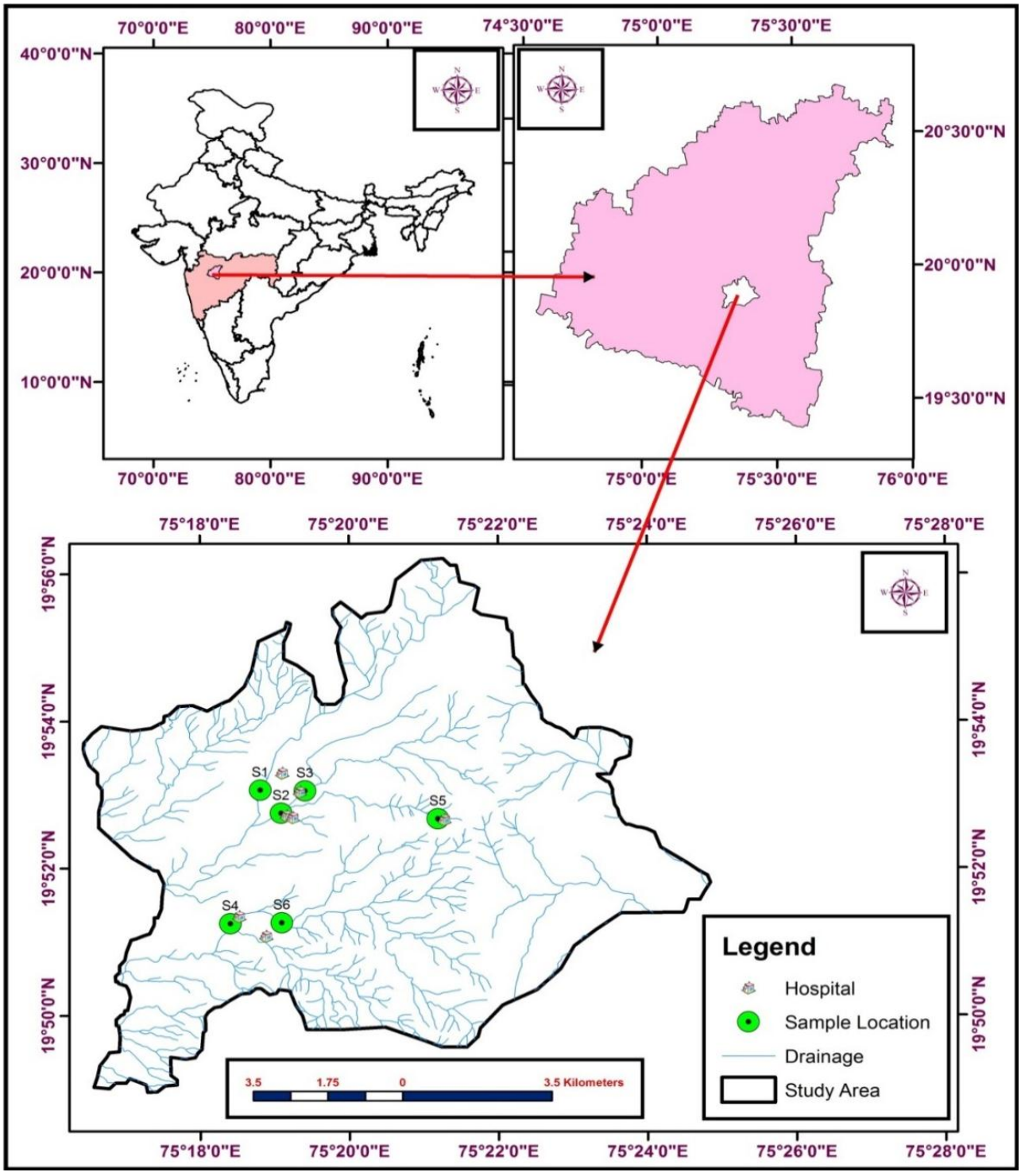


Figure 1. Hospital sewage sample location map of Aurangabad Urban Area, Maharashtra India.

ARI = y/nx

Where, y is actual number of resistance microbes in the sample, n is the number of isolates tested and x is the total antibiotics tested in sensitivity test. Generally, ARI index is directly proportional to the prevalence of antibiotic resistance as per sample collection sites.

RESULTS AND DISCUSSION

Biochemical analysis

Further, by using the biochemical tests and referring to

Table 1. Biochemical tests and identity.

Site	Sample ID	GS	Biochemical test and results								Sugar fermentation test results					Bacterial identity
			CO	CI	IN	CT	OD	MR	VP	DN	Glucose	Sucrose	Lactose	Maltose	Mannitol	
	S11	-	-	+	-	+	-	-	+	-	+	+	+	+	+	*Klebsiella pneumoniae*
	S12	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
S1	S13	-	-	+	-	+	-	-	+	-	+	+	+	+	+	*Klebsiella pneumoniae*
	S14	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S15	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S21	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S22	-	-	+	-	+	-	-	+	-	+	+	+	+	+	*Klebsiella pneumoniae*
S2	S23	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S24	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S25	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S31	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S32	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
S3	S33	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S34	-	-	+	-	+	+	-	-	-	-	-	-	-	+	*Pseudomonas species*
	S35	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S41	-	-	+	-	+	-	-	+	-	+	+	+	+	+	*Klebsiella pneumoniae*
	S42	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
S4	S43	-	-	+	-	+	-	-	+	-	+	+	+	+	+	*Klebsiella pneumoniae*
	S44	-	-	+	-	+	+	-	-	-	-	-	-	-	+	*Pseudomonas species*
	S45	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S51	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S52	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
S5	S53	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S54	-	-	+	-	+	-	-	+	-	+	+	+	+	+	*Klebsiella pneumoniae*
	S55	-	-	+	-	+	-	-	+	-	+	+	+	+	+	*Klebsiella pneumoniae*
	S61	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S62	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
S6	S63	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S64	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*
	S65	-	ND	-	+	+	-	+	-	-	+	V	+	+	+	*Escherichia coli*

GS: Gram staining, CI: Citrate, IN: Indole, CT: Catalase, OD: Oxidase; MR: Methyl red; VP: Voges–Proskauer; - Negative; +: Positive; CO: coagulase, DN: DNase, ND: No data; V= variable.

the Bergey's Manual of Systematic Bacteriology, all isolates were identified (Table 1). In biochemical tests carried out for 48 isolates, we found a total of 6 different types of bacteria out of which 3 were Gram negative and 3 were Gram positive namely, *Klebsiella pneumoniae,*

Table 2. Results of staining, biochemical tests and identity of isolates selected for further study.

Site	Sample ID	GS	Biochemical test and results								Sugar fermentation test results					Bacterial identity
			CO	CI	IN	CT	OD	MR	VP	DN	Glucose	Sucrose	Lactose	Maltose	Mannitol	
S1	S16	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	S17	+	+	-	-	-	-	ND	+	ND	+	+	+	+	+	*Enterococcus faecalis*
	S18	+	+	-	-	-	-	ND	+	ND	+	+	+	+	+	*Enterococcus faecalis*
S2	S26	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	S27	+	-	ND	-	-	-	+	-	+	+	+	+	+	-	*Streptococcus sp.*
	S28	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
S3	S36	+	-	ND	-	-	-	+	-	+	+	+	+	+	-	*Streptococcus pyogenes*
	S37	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	S38	+	+	-	-	-	-	ND	+	ND	+	+	+	+	+	*Enterococcus faecalis*
S4	S46	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	S47	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	S48	+	+	-	-	-	-	ND	+	ND	+	+	+	+	+	*Enterococcus faecalis*
S5	S56	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	S57	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	S58	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
S6	S66	+	+	+	-	+	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*
	S67	+	+	-	-	-	-	ND	+	ND	+	+	+	+	+	*Enterococcus faecalis*
	S68	+	+	-	-	-	-	ND	+	ND	+	+	+	+	+	*Enterococcus faecalis*

CI: Citrate, IN: Indole, CT: Catalase, OD: Oxidase; MR: Methyl red; VP: Voges–Proskauer; - Negative; +: Positive; CO: coagulase, DN: Dnase, ND: No data; V= variable.

Escherichia coli, Pseudomonas species, Staphylococcus aureus, Enterococcus faecalis and *Streptococcus pyogenes.* Among all bacteria obtained, Gram negative *E. coli* and Gram positive bacteria *S. aureus* were the most prevalent bacteria found at all sites.

Antibiotic resistance pattern

Based on the zone of inhibition (mm) obtained, antibiotic sensitivity of isolates as per the guidelines given by Clinical and Laboratory Standards Institute (CLSI, 2009) mentioned in Table 3 (Gram negative bacteria) and Table 4 (Gram positive bacteria), the isolates are divided into 2 types, viz: resistant and sensitive. Isolates with intermediate phenotypes obtained in AST were considered as antibiotic resistant bacteria. Among all the antibiotics tested, highest level of resistance was observed for beta lactam class [85%], then for Tetracycline [58%], Cephalosporin [58%], quinolones [52%] and gentamycin [45%] (Figure 2). *E. coli* was the most prevalent bacteria showing antibiotic resistance to all tested antibiotics.

Multiple antibiotic resistance pattern

Further MAR index values were calculated for all the isolates. MAR index values indicate the number of antibiotics to which the isolate is showing resistance. When the MAR index value is >0.2, the bacteria is considered as multiple antibiotic resistant bacteria. From the MAR index values we can predict that around 41 out of 48 isolates show multiple antibiotic resistance and only four are not MAR (Tables 5 and 6). Some of the isolates S11, S22, S34, S35, S38, S54, S55, S56 and S64 were found to be resistant to all the

Table 3. Results of antibiotic sensitivity test of Gram negative isolates.

Site	Sample ID	Zone of Inhibition (mm)				
		Beta lactams [Amp]	Tetracycline [Tet]	Cephalosporin	Quinolones [Cip]	Aminoglycosides
S1	S11	10	14	12	11	11
	S12	20	10	23	15	09
	S13	19	22	13	14	10
	S14	23	12	10	24	20
	S15	29	14	11	26	28
S2	S21	18	22	20	17	24
	S22	14	11	08	10	12
	S23	29	18	00	28	24
	S24	22	10	10	26	18
	S25	20	25	18	14	25
S3	S31	06	22	23	27	26
	S32	09	24	13	15	11
	S33	29	22	11	26	28
	S34	12	14	10	13	10
	S35	19	12	08	10	09
S4	S41	20	11	10	30	25
	S42	30	18	17	18	14
	S43	20	18	09	13	08
	S44	21	11	19	27	22
	S45	22	12	13	22	19
S5	S51	12	11	26	27	18
	S52	14	09	11	24	06
	S53	24	12	24	24	22
	S54	19	14	13	14	11
	S55	10	13	10	11	10
S6	S61	16	09	08	08	20
	S62	12	05	07	09	22
	S63	30	11	10	11	23
	S64	10	14	13	10	10
	S65	08	25	19	15	21

antibiotics tested in the present study while the sample S48 and S58 showed sensitivity to all the antibiotics used. Mainly, *K. pneumoniae* bacteria showed resistance to most of the tested antibiotics.

ARI index

ARI index indicates the prevalence of antibiotic resistant and sensitive bacteria as per the locations. In the present study, it was observed from the ARI (Table 7) that beta lactam resistant bacteria are more prevalent in nature as compared to other antibiotics at all the sample collection sites. The prevalence of antibiotic resistant bacteria is shown in Graph 1. Beta lactam antibiotic resistant bacteria are the more prevalently occurring bacteria in nature. Bacteria obtained from the site 1 appear more resistant to all the antibiotics as compared to other sites.

Conclusion

The present study on the hospital sewage water collected from six different locations of Aurangabad, Maharashtra, India has shown .*E coli* as the most prevalent antibiotic resistant bacteria. We obtained Gram negative bacteria more abundantly in hospital sewage samples. Isolates number 48 and 41 showed >0.2 MAR indices and hence, it is considered to have the potential to cause human infections. Most of the isolates showed multiple antibiotic resistance. Among tested antibiotics, beta lactam group antibiotic resistant bacteria were found to be most prevalent and comparatively less number of isolates was resistant to gentamycin. Therefore, there is need to prepare effective guidelines for judicious use of antibiotics and release of hospital sewage directly into the water bodies, in order to avoid the spread of bacterial multiple antibiotic resistances.

Table 4. Results of antibiotic sensitivity test of Gram positive isolates.

Site	Sample ID	Zone of Inhibition (mm)				
		Beta Lactams [Amp]	Cephalosporin [Cx]	Quinolones [Cip]	Aminoglycosides [Gen]	Tetracycline [Tet]
S1	S16	20	16	14	14	11
	S17	18	20	22	10	20
	S18	19	22	12	09	10
S2	S26	18	23	18	10	15
	S27	15	20	22	19	13
	S28	06	06	32	20	06
S3	S36	08	19	24	24	09
	S37	12	11	13	10	25
	S38	13	10	10	24	09
S4	S46	07	08	30	25	11
	S47	20	30	30	11	31
	S48	32	25	22	23	25
S5	S56	13	11	14	11	09
	S57	12	11	30	22	20
	S58	29	22	30	30	21
S6	S66	12	18	`13	16	22
	S67	20	19	21	23	22
	S68	06	20	11	18	20

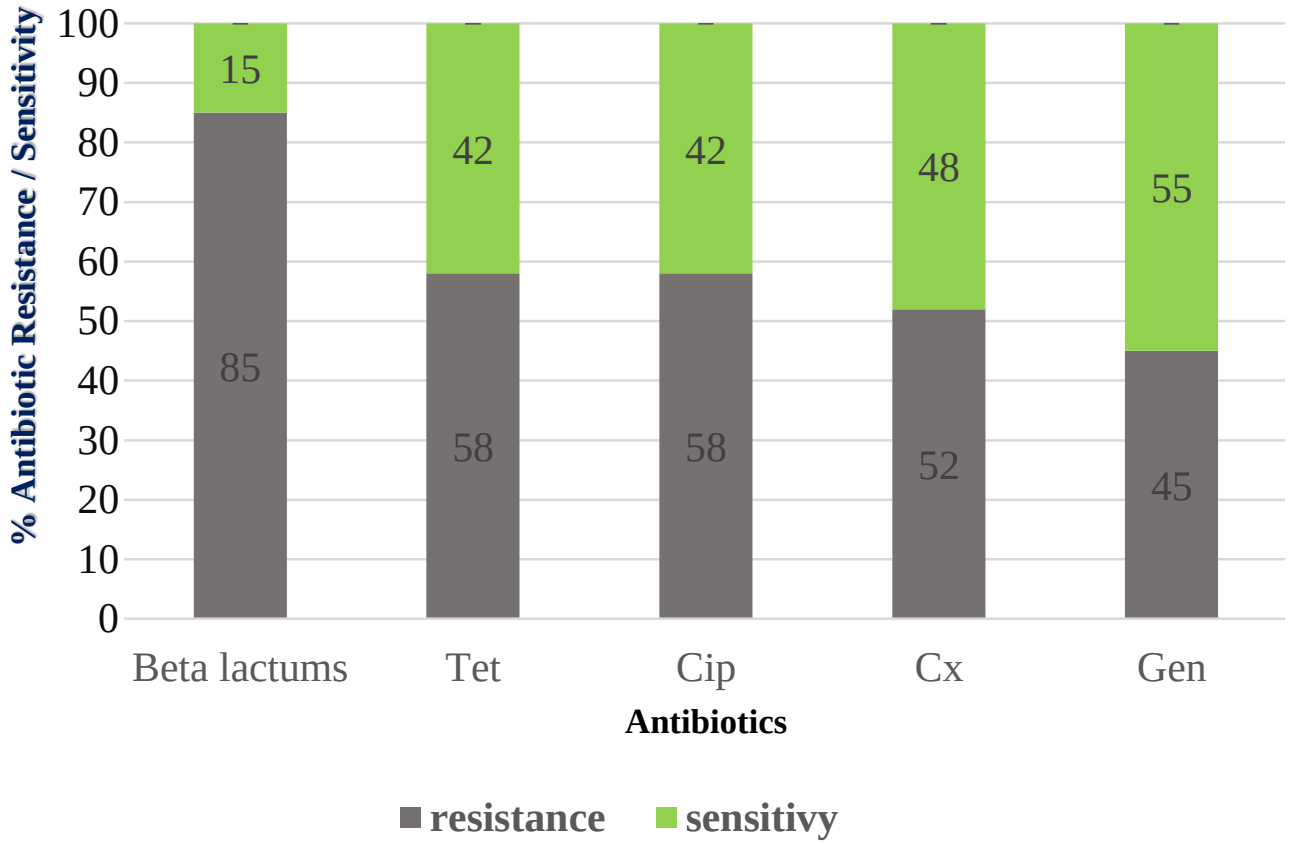


Figure 2. Prevalence of antibiotic resistance.

Table 5. MAR index values of Gram negative isolates.

Site	Sample ID	Antibiotic resistance status					MAR
		Beta lactams [Amp]	Tetracycline [Tet]	Cephalosporin [Cx]	Quinolones [Cip]	Aminoglycoside [Gen]	
S1	S11	R	R	R	R	R	1
	S12	R	R	S	R	R	0.8
	S13	R	S	R	R	R	0.8
	S14	R	R	R	S	S	0.6
	S15	S	R	R	S	S	0.4
S2	S21	R	S	S	R	S	0.4
	S22	R	R	R	R	R	1
	S23	S	S	R	S	S	0.2
	S24	R	R	R	S	R	0.8
	S25	R	S	S	R	S	0.4
S3	S31	R	S	S	S	S	0.2
	S32	R	S	R	R	R	0.8
	S33	S	S	R	S	S	0.2
	S34	R	R	R	R	R	1
	S35	R	R	R	R	R	1
S4	S41	R	R	R	S	S	0.6
	S42	S	S	S	S	R	0.2
	S43	R	S	R	R	R	0.8
	S44	R	R	S	S	S	0.4
	S45	R	R	R	S	S	0.6
S5	S51	R	R	S	S	S	0.4
	S52	R	R	R	S	R	0.8
	S53	R	R	S	S	S	0.4
	S54	R	R	R	R	R	1
	S55	R	R	R	R	R	1
S6	S61	R	R	R	R	S	0.8
	S62	R	R	R	R	S	0.8
	S63	S	R	R	R	S	0.6
	S64	R	R	R	R	R	1
	S65	R	S	S	R	S	0.4

Table 6. MAR index values of Gram positive isolates.

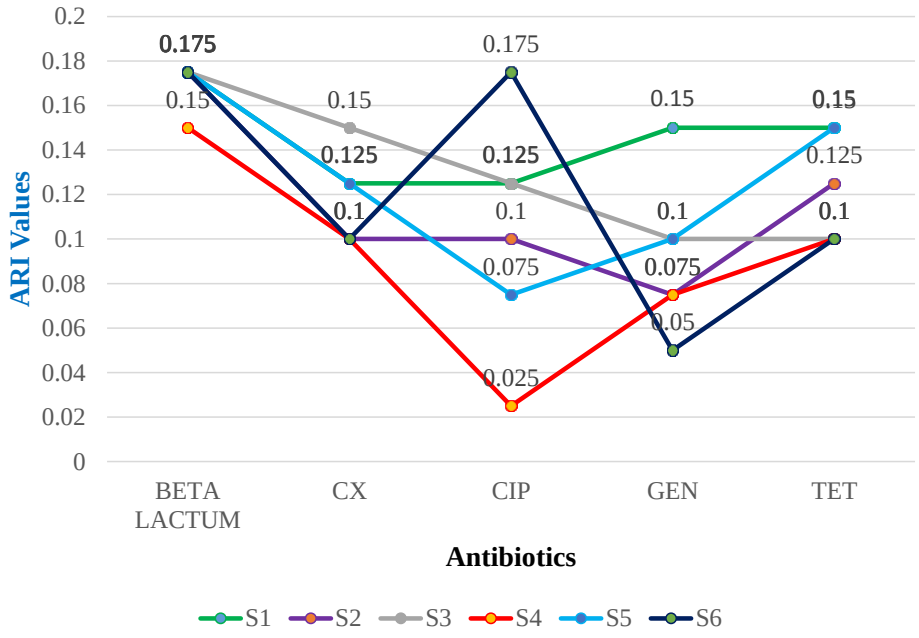
Site	Sample ID	Zone of inhibition (mm)					MAR
		Beta Lactams [Amp]	Cephalosporin [Cx]	Quinolones [Cip]	Aminoglycoside [Gen]	Tetracycline [Tet]	
S1	S16	R	I	R	I	R	0.6
	S17	R	S	S	R	S	0.4
	S18	R	S	R	R	R	0.8
S2	S26	R	S	I	R	I	0.4
	S27	R	S	S	S	R	0.4
	S28	R	R	S	S	R	0.6
S3	S36	R	S	S	S	R	0.4
	S37	R	R	R	R	S	0.8
	S38	R	R	R	S	R	1
S4	S46	R	R	S	S	R	0.6
	S47	R	S	S	R	S	0.4
	S48	S	S	S	S	S	0
S5	S56	R	R	R	R	R	1

Table 6. Contd.

	S57	R	R	S	S	S	0.4
	S58	S	S	S	S	S	0
	S66	R	S	R	I	S	0.4
S6	S67	R	S	S	S	S	0.2
	S68	R	S	R	S	S	0.4

Table 7. ARI index.

Site	ARI Values				
	Beta Lactams [Amp]	Cephalosporin [Cx]	Quinolones [Cip]	Aminoglycosides [Gen]	Tetracycline [Tet]
S1	0.175	0.125	0.125	0.15	0.15
S2	0.175	0.1	0.1	0.075	0.125
S3	0.175	0.15	0.125	0.1	0.1
S4	0.15	0.1	0.025	0.075	0.1
S5	0.175	0.125	0.075	0.1	0.15
S6	0.175	0.1	0.175	0.05	0.1



Graph 1. Prevalence of antibiotic resistance as per site of sample collection.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adefisoye MA, Okoh AI (2017). Ecological and public health implications of the discharge of multidrug-resistant bacteria and physicochemical contaminants from treated wastewater effluents in the Eastern Cape, South Africa. Water 9(8):562.

Biyela PT, Lin J, Bezuidenhout CC (2004). The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. Water Science and Technology 50(1):45-50.

Clinical and Laboratory Standards Institute (CLSI) (2011). Performance standards for antimicrobial susceptibility testing, 21st informational supplement M100S21. CLSI, Wayne, Pa.

Clinical and Laboratory Standards Institute (CLSI) (2009). Performance Standards for Antimicrobial Susceptibility Testing M100-S19;

Nineteenth Informational Supplement. Vol. 29, Clinical and Laboratory Standards Institute, Wayne.
De Kraker ME, Stewardson AJ, Harbarth S (2016). Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? PLoS Medicine 13(11):e1002184.
De Kraker MEA, Davey PG, Grundmann H (2011). Mortality and Hospital Stay Associated with Resistant *Staphylococcus aureus* and *Escherichia coli* Bacteremia: Estimating the Burden of Antibiotic Resistance in Europe. PLoS Medicine 8(10):e1001104.
Forbes BA, Sahm DF, Weissfeld AS (2007). Bailey and Scott's diagnostic of microbiology". 12th Edition, Mosby (Elsevier), USA.
Government of India (2017). National Action Plan on Antimicrobial Resistance (NAP-AMR) 2017-2021. [accessed on May 15, 2019] Available from: http://www.searo.who.int/india/topics/antimicrobial_resistance/nap_amr.pdf .
Holt JG (1984). Bergey's Manual of Systematic Bacteriology". Williams and Wilkins, Baltimore.
Krumperman PH (1983). Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Applied and Environmental Microbiology 46(1):165-170.
Mckenzie R, Buckle J, Wegelin W, Meyer N (2003). Water demand management cookbook, rand water in collaboration with WRP, managing water for African Cities UN Habitat Programme, New York. Medical Bulletin 68:199-208.
Mukhopadhyay C, Vishwanath S, Eshwara VK, Shankaranarayana SA, Sagir A (2012). Microbial quality of well water from rural and urban households in Karnataka, India: a cross-sectional study. Journal of infection and Public Health 5(3):257-262.
Mulamattathil SG, Bezuidenhout C, Mbewe M, Ateba CN (2014). Isolation of environmental bacteria from surface and drinking water in Mafikeng, South Africa, and characterization using their antibiotic resistance profiles. Journal of Pathogens 10(4):1-11.
Mulamattathil SG, Esterhuysen H.A, Pretorius PJ (2000). Antibiotic-resistant Gram-negative bacteria in a virtually closed water reticulation system. Journal of Applied Microbiology 88(6):930-937.
Nandi S, Mandal S (2016). Bacteriological profiling of commercially available eye cosmetics and their antibiotic susceptibility pattern. Translational Biomedicine 7(3):1-8.
Obi CL, Potgieter N, Bessong PO, Matsaung G (2002). Assessment of the microbial quality of river water sources in rural Venda communities in South Africa. Water SA 28(3):287-292.
Sidhu S, Malhotra S, Devi P, Gupta A (2016). Bacteriological analysis of the drinking water from different schools in Northern India: a concern in developing countries. International Journal of Medical Resarch and Review 4(4):630-634.
Smith E, Lichten CA, Taylor J, MacLure C, Lepetit L, Harte E (2016). Evaluation of the action plan against the rising threats from antimicrobial resistance. Final Report. European Commission. 2016. [accessed on May 15, 2019]. Available from: https://www.ec.europa.eu/health/amr/sites/amr/files/amr_final-report_2016_rand.pdf .
World Health Organization (WHO) (2011). Regional office for South-East Asia. Jaipur declaration on antimicrobial resistance. 2011. [accessed on May 15, 2019]. http://www.who.int/iris/handle/10665/205397.