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Trends in Antimicrobial Resistance of Bacterial Isolates Circulating in Sewage Waters of Aligarh Region over a Period of 14 Years

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Abstract: Antibacterial-resistant pathogens are serious threat worldwide. The presence of drug-resistant bacteria in environmental samples has important implications for people living in the area who are reliant on public water and sanitation facilities. Detection of faecal indicator bacteria in water provides a very sensitive method of quality assessment. This study was designed to compare the prevalence of gram-negative bacteria circulating in sewer lines of Aligarh region at an interval of every four to five years. This study was conducted in the department of Microbiology, JN Medical College and Hospital over a period of fourteen years. Trends of antimicrobial susceptibility were assessed over this period. Isolates resistant to any of the 3rd generation cephalosporins were also evaluated for the presence of extended spectrum β-lactamases, Amp-C and metallo-β-lactamases. *Escherichia coli* was the commonest coliform isolated throughout the 14-year study period. Polymicrobial growth was more common in 2005 and 2013. Increase in the antimicrobial resistance was noted among the bacterial strains isolated in the last eight years of study. Isolation of ESBL and Amp-C producing bacterial isolates in sewage waters was the most alarming finding. Circulation of antimicrobial resistant bacteria in the environment is an alarming indicator of the rampant use of antibiotics. It is suggested that periodic surveys should be done to study the prevalence and susceptibility pattern of environmental water samples.

Key words: Sewage water, coliform, drug resistance, ESBL, Amp-C, MBL.

Introduction

Pure and safe drinking water is a basic necessity for any sort of life in a region. According to the WHO estimates, more than three million children below age 5 die annually from diarrheal diseases contracted through drinking water in developing world (WHO, 2007). The major pathogenic bacteria responsible for water-borne diseases are spread by the feco-oral route, in which water may play a significant role. Even if the source of water supply and its treatment are of high standard, water pollution may still occur as often happens, due to corrosion of pipelines, leaky joints and cross connections between water supplies and sewage drainage pipes (Shukla et al., 2010).

Detection of faecal indicator bacteria in water provides a very sensitive method of quality assessment. Indicator organism survive better and longer than pathogens and may be easily detected by standard laboratory techniques. The coliform group of bacteria is a criterion for the degree of pollution and thus of sanitary quality. The coliforms can mediate the transfer of antibiotic resistance in aquatic environment where polluted surface waters can be considered as a point

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source for spreading of the antibiotic resistance via many possible routes which may ultimately reach humans. The presence of drug resistant bacteria in environmental samples has important implications for people living in the area who are reliant on public water and sanitation facilities.

Antibacterial-resistant pathogens are serious threat for worldwide hospitalized patients. Rapid expanding multidrug resistant bacteria is a major public health problem (Green et al., 2011; Jean et al., 2009; Fernández et al., 2011). Not all patients infected with the resistant pathogens have history of hospital admission in India, and extended spectrum β-lactamases are known to be circulating in Indian community (Walsh, 1990). $bla_{\text{NDM-1}}$ is a recently described novel plasmid-mediated resistance determinant that confers resistance to carbepenems. NDM-1 gene has been discovered in patients in more than a dozen countries and has been found to be widely harboured outside hospitals in India, and in surface waters and sewage.

Aims and Objectives

This study was designed to compare the prevalence of gram negative bacteria circulating in sewer lines of Aligarh region at an interval of every four to five years. Trends of antimicrobial susceptibility profile were assessed over this period. Isolates resistant to any of the 3rd generation cephalosporins were also evaluated for the presence of extended spectrum β-lactamases (ESBL), Amp-C and metallo-β-lactamases (MBL).

Methods

The study was conducted in the years 1999, 2005 and 2013 in the Department of Microbiology, JN Medical College and Hospital. With all aseptic precautions environmental samples, in an approximate volume of 15 ml, were collected from different areas of Aligarh city.

Multiple Tube Technique

Most probable number (MPN) of *E. coli* was calculated by the five-tube procedure by using Macconkey's broth (Collee et al., 2006). The samples were plated on Teepol lactose agar at 37°C for 18-24 hours and on Eosin methylene blue agar plates. The isolates were identified by standard laboratory procedures (Collee et al., 2006).

Antibiotic Susceptibility Testing

Sensitivity to appropriate antibiotics was determined by the Kirby Bauer Disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI, 2008) guidelines, using the commercially available antibiotic discs from HiMedia (Mumbai, India). For testing the susceptibility profile of *E. coli* isolated in the year 1999 the antimicrobials used were: amikacin (30 µg), ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefaloridine (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg, 1.25/23.75 µg), pefloxacin (5 µg), piperacillin (100 µg) and tetracycline (30 µg).

The antimicrobials tested for *Enterobacteriaecae* in the years 2005 and 2013 were amikacin (30 µg), ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefaloridine (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), pefloxacin (5 µg), piperacillin (100 µg) and tetracycline (30 µg) along with newly added antimicrobials like gentamicin (10 µg), ofloxacin (5 µg), ceftriaxone (30 µg), cefixime (15 µg), cefoperazone (75 µg), cefpodoxime (30 µg), cefepime (30 µg), cefoperazone sulbactum (75/75 µg) and imipenem (10 µg). For *Pseudomonas* isolates antimicrobials used were gentamicin (10 µg), amikacin (30 µg), ticarcillin (75 µg), pipercillin (100 µg), pipercillin tazobactum (100/10 µg), ceftazidime (30 µg), polymixin (300 µg), colistin (10 µg) and imipenem (10 µg).

Detection of ESBL, Amp C and MBL

Screening of possible ESBL production was done by using ceftriaxone (30 µg) and cefoperazone (75 µg). Those isolates with zone diameters less than 25 mm for ceftriaxone and less than 22 mm for cefoperazone were subsequently confirmed for ESBL production. Confirmation was done by noting the potentiation of the activity of cefoperazone in the presence of cefoperazone sulbactum (Rizvi et al., 2009). Detection of AmpC betalactamase was done on isolates resistant to ceftriaxone (30 µg), cefixime (15 µg), cefoperazone (75 µg) and cefoperazone sulbactum (75/75 µg). Induction of AmpC synthesis was based on the disc approximation assay using imipenem as inducer (Rizvi et al., 2009). Detection of MBL was done by Hodge test and Double Disc synergy test using EDTA. The method was as described by Lee et al. (2001).

Results

During the year 1999, 20 *E. coli* strains were isolated from the sewage. On antimicrobial susceptibility testing they were found to be 100% sensitive to ciprofloxacin, corimoxazole, chloramphenicol, pefloxacin, piperacillin and amikacin. The susceptibility to tetracycline (95%), cefotaxime (95%), ceftazidime (65%) and cefaloridine (60%) was lower as compared to the above mentioned drugs (Table 1).

Table 1: Antimicrobial susceptibility pattern of bacterial isolates from sewage over a period of 14 years

Period		*Sensitivity profile of antimicrobials tested No. (%)*																							
		Amikacin	*Cefepime*	*Cefoperazone*	*Cefoperazone sulbactum*	*Ceftriaxone*	*Cefexime*	*Cefpodoxime*	*Gentamycin*	*Ofloxacin*	*Ampicillin*	*Cefotaxime*	*Ceftazidime*	*Cefaloridine*	*Chloramphenicol*	*Ciprofloxacin*	*Cotrimoxazole*	*Pefloxacin*	*Piperacillin*	*Tetracycline*	*Imipenem*	*Piperacillin tazobactum*	*Colistin*	*Ticarcillin*	*Polymixin*
2013	Enterobacteriaceae (23)	22 (95.6)	21 (91.3)	20 (86.9)	22 (95.6)	21 (91.3)	19 (82.6)	20 (86.9)	21 (91.3)	19 (82.6)	60.8 (14)	20 (86.9)	13 (56.5)	12 (52.2)	-	19 (82.6)	-	20 (86.9)	20 (86.9)	-	23 (100)	22 (95.6)	-	-	-
	Nil fermenters (14)	12 (85.7)	12 (85.7)	-	-	-	-	-	-	-	-	-	12 (85.7)	-	-	-	-	-	11 (78.6)	-	14 (100)	12 (85.7)	13 (92.8)	11 (78.6)	14 (100)
2005	Enterobacteriaceae (31)	30 (96.7)	29 (93.5)	27 (87.1)	30 (96.7)	29 (93.5)	27 (87.1)	27 (87.1)	30 (96.7)	27 (87.1)	22 (70.9)	27 (87.1)	19 (61.3)	19 (61.3)	-	29 (93.5)	-	30 (96.7)	30 (96.7)	-	31 (100)	31 (100)	-	-	-
	Nil fermenters (8)	7 (87.5)	7 (87.5)										7 (87.5)	-	-	-	-	-	7 (87.5)	-	8 (100)	8 (100)	8 (100)	7 (87.5)	8 (100)
1998	Escherichia coli (20)	20 (100)	-	-	-	-	-	-	-	-	20 (100)	19 (95)	13 (65)	12 (60)	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)	19 (95)	-	-	-	-	-

In 2005, 21 sewage samples were collected, of which 11 showed monomicrobial, while 10 showed polymicrobial growth. *E. coli* 16 (47.6%) was the commonest bacterium isolated from these samples. The other bacteria isolated were *Citrobacter* 8 (22.8%), *Klebsiella* 5 (14.3%), *Pseudomonas* 4 (11.4%) and *Proteus* 2 (5.7%) (Figure 1). The sensitivity pattern in this year was as follows: piperacillin (96.7%), cefotaxime (87.1%), ciprofloxacin (93.5%), ampicillin (70.9%), ceftazidime (61.3%) and cefaloridine (61.3%). The new drugs added in 2005 showed enhanced sensitivity to these isolates: amikacin (96.7%), cefepime (93.5%), cefoperazone (87.1%), cefoperazone-sulbactum (96.7%), ceftriaxone (93.5%), cefexime (87.1%), cefpodoxime (87.1%), gentamycin (96.7%), ofloxacin (87.1%) and imipenem (100%) (Table 1).

In the year 2013, the test results showed the presence of coliforms in all the 18 sewage samples. On culture 6 (33.3%) specimens were found to harbour single bacterium while 12 (66.7%) samples showed the growth of more than one organism. Out of a total of 38 bacteria isolated from these samples, *Escherichia coli* was found to be the commonest (42.1%), followed by *Pseudomonas* species (36.8%), *Proteus* 3 (7.9%), *Citrobacter* 2 (5.3%), *Klebsiella* 2 (5.3%) and 1 (2.6%) *Acinetobacter* (Figure 2). In the year 2013, there was a marked decrease in the sensitivity to the antimicrobials tested. None of the drugs tested in the year 1999 was found to be 100% sensitive for any of the isolates tested.

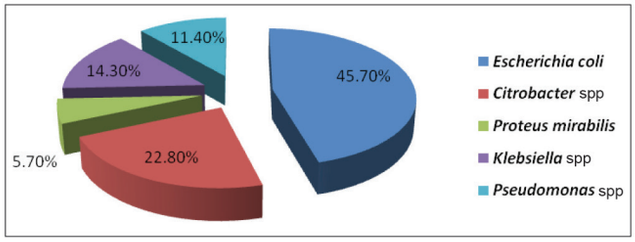

Figure 1: Gram negative bacteria isolated from sewage samples of Aligarh region in 2005.

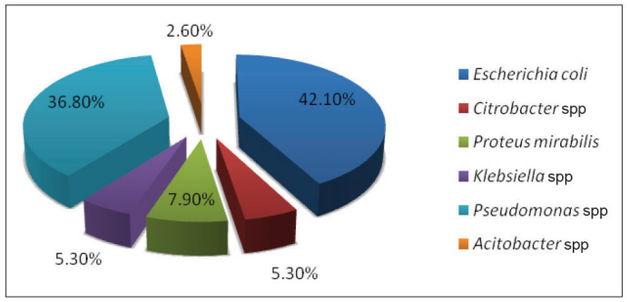

Figure 2: Gram negative bacteria isolated from sewage samples of Aligarh region in 2013.

The sensitivity pattern in decreasing order was found to be as follows: piperacillin (86.9%), cefotaxime (86.9%), ciprofloxacin (82.6%), ampicillin (60.8%), ceftazidime (56.5%) and cefaloridine (52.2%). The new drugs added in 2013 showed enhanced sensitivity to these isolates: amikacin (95.6%), cefepime (91.3%), cefoperazone (86.9%), cefoperazone sulbactum (95.6%), ceftriaxone (91.3%), cefexime (82.6%), cefpodoxime (86.9%), gentamycin (91.3%), ofloxacin (82.6%) and imipenem (100%) (Table 1).

Amongst the strains tested in 1999, neither ESBL nor Amp-C β-lactamase was detected. However, in 2005, out of the 39 isolates tested 4 (10.2%) were found to be ESBL producers and 1(2.5%) isolate was Amp-C producer. In 2013, 3 (8.1%) isolates showed ESBL production, while there was an increase in Amp-C producers from just 2.5% in 2005 to 8.1% in 2013. However, MBL was not detected in any of the isolates throughout the study period.

Discussion

The occurrence and spread of antibiotic resistant bacteria are imperative public health problems worldwide and aquatic ecosystems are a recognized reservoir for the resistant bacterial strains (Baquero et al., 2008; Cooke, 1975). Ever increasing urbanization, industrialization and agriculture have caused microbial as well as physico-chemical contamination of natural water sources. According to WHO (2007), approximately 1.7 million deaths per year can be attributed to unsafe water supplies. In India surface water and ground water are the main sources of drinking water. Contamination of drinking water supply is becoming an increasingly serious problem particularly in urban areas (Knepper et al., 2004). Sewage contaminated drinking water is the major source of several water-borne diseases. This study was therefore undertaken to evaluate the bacterial flora in the sewage waters of Aligarh and its antimicrobial resistance over a period of 14 years.

In our study *E. coli* was the most prevalent coliform (100% in 1999, 47.6% in 2005 and 42.1% in 2013) in the sewage water throughout the 14-year study period. However, in the later years i.e., 2005 and 2013, the sewage flora was found to harbour diverse bacterial strains like *Citrobacter, Klebsiella, Pseudomonas,* and *Proteus* which were not isolated in 1999. More alarming was the increase in the antimicrobial resistance of these strains in the last eight years of study. This may be because of the increased and indiscriminate use of antimicrobials and more importantly the ease over the

counter availability of these drugs. Inhibition of normal gastro-intestinal flora by antibacterial therapies results in increased replication of resistant microorganisms (Fallah et al., 2010).

The presence of ESBL (10.2% in 2005, 8.1% in 2013) and Amp-C (2.5% in 2005, 8.1% in 2013) producing bacteria in the sewage waters is another warning for an impending catastrophe. The fecal flora harbouring these resistance mechanisms can reach the potable waters, which is a matter of grave concern (Mohd. et al., 2012). Bacteria harbouring NDM-1 have been reported from both clinical and fecal specimens (Yong et al., 2009; Kumaraswamy et al., 2010; Nordmann et al., 2011). They have recently been described in the environmental samples from the capital city of India (Walsh et al., 2011) indicating a reservoir for future infections. However, in our study, none of the isolates were MBL producers. However, the presence of ESBL and AmpC producing bacteria in the sewage specimens is in itself an indication to become more vigilant.

Conclusion

It is suggested that periodic surveys should be done to study the prevalence and susceptibility pattern of environmental water samples. Combined environmental interventions, including water quality improvement, household and community sanitation, have a powerful impact on reducing serious diarrheal diseases in the community. Circulation of antimicrobial resistant bacteria in the environment is an alarming indicator of the rampant use of antibiotics. Therefore, stress should be given on the restrained and rationale use of antimicrobials. Proper execution of antibiotic prescribing and antibiotic resistance monitoring policies is the need of the hour.

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