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Original Research Article

Antibiotic Resistant Enterobacteriaceae in Healthy Gut Flora of a Semi Urban Community in Siddipet

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Abstract:

Introduction: Antibiotic resistance is concerningly on the rise in our communities and hospitals, increasing the risk of treatment failure and recurrent infections. The human healthy intestinal gut flora has been altered by the improper use of antibiotics, creating a reservoir of microbes resistant to antibiotics known as the "gut resistome.

Methodology: From 60 healthy Siddipet residents, 60 stool samples were taken. It was then processed using standard laboratory procedures to isolate two aerobic enteric bacteria, Klebsiella pneumoniae and Escherichia coli. The Clinical and Laboratory Standards Institute-recommended Kirby Bauer Disc Diffusion method was used for antibiotic susceptibility testing.

Results: Out of the 60 subjects, 100% had E. Coli, 80% had K. pneumoniae, and 20% had a combination of both E. Coli and K. pneumoniae isolates. These two microbes have demonstrated resistance to multiple antibiotic classes. Cephalosporins exhibited the highest level of resistance (100%) followed by fluoroquinolones (50%) and aminoglycosides (43%).

Conclusion: It is concerning to find commensal organisms with resistance genes. There's a chance that this will spread and might manifest them when antibiotic pressure is present. To determine the actual prevalence of MDR in the community, more research involving environmental sampling and a correlation of local prescription patterns must be conducted.

Keywords: ESBL, AmpC, MDR, Diagnosis, Surveillance.

Introduction

One of the wonders of contemporary medicine is the antibiotic. They have been vital tools in the fight against infections and sepsis for the past century, having saved billions of lives. But within a century of the first antibiotic's discovery, the emergence of antibiotic resistance proves to be a serious public health issue in terms of mortality and financial loss.

Antimicrobial resistance (AMR) alone is expected to kill 10 million more people by 2050 than cancer and traffic accidents put together, with 700,000 people losing their lives to AMR each year. India, also known as "the AMR capital of the world," leads the globe in the overall amount of antibiotics consumed for human purposes. Antibiotic resistance is concerningly on the rise in our communities and hospitals, increasing the risk of treatment failure and recurrent infections. The uncontrolled use of antibiotics in India has resulted in a confluence of medical, agricultural, veterinary, social, and environmental problems. Since India is the world's largest milk producer and has enormous potential in the food animal business, antimicrobial drugs like vancomycin and colistin are frequently employed to boost output. Faeces and urine carry around half of the antibiotics that are consumed out of the body unaltered. For many years, open defecation has been a common practice in India. This practice allows antibiotics or their remnants to leach into the environment through soil and water.

The human healthy intestinal gut flora has been altered by the improper use of antibiotics, creating a reservoir of microbes resistant to antibiotics known as the "gut resistome [1]. The disruption of the gut microbiome caused by selection pressure enables these organisms to act as opportunistic infections. The issue is made worse by the community's growing presence of these resistant bacteria, which raises the possibility of cross-transmission [2, 3]. High-end medications like

carbapenems and polymyxins are required to treat hospital-acquired infections due to an increase in pathogen resistance brought on by inadequate antimicrobial stewardship in hospitals and a lack of community knowledge [4, 5]. India recently created a national action plan to address the threat of rising antibiotic resistance in the nation. Community level efforts to address the problem of resistant gut flora have not yet reached the point where ambient solutions are implemented.

Most faecal carriage data comes from hospitalized patients who are receiving antibiotic therapy. Information regarding the carriage and dissemination of resistant strains within communities is scarce. The purpose of this study was to ascertain whether the guts of a representative sample of healthy persons living in a semiurban area near Government General Hospital Siddipet were home to antibiotic-resistant bacteria.

Methodology:

This was a prospective cross- sectional study carried out during March 2023 to May 2023 at tertiary care hospital, Siddipet, Telangana. The study protocol was reviewed and approved by the institute's scientific review committee.

Study Subjects: In all, 60 healthy persons of both genders, aged 18 to 35, participated in the study. All patients with any illness that would have an impact on the endogenous flora, such as a history of antibiotic use within the last three months or a history of hospitalization within the previous year, were excluded.

Sample: From 60 healthy Siddipet residents, 60 stool samples were collected in sterile containers. A wooden spatula to gather the faeces and a wide mouth, disposable plastic container sealed against leaks were given to each participant. These were delivered to the lab that same day. For the benefit of the participants, the specimen was inspected both macroscopically and microscopically for ova and cysts. It was then processed using standard laboratory procedures to isolate two aerobic enteric bacteria, Klebsiella pneumoniae and Escherichia coli, because of their propensity to carry resistance genes. After being inoculated on MacConkey agar, stool samples were incubated for 24 hours at 37 °C. Using common biochemical reactions, Escherichia coli and Klebsiella were found in every stool sample investigated.

Testing for antibiotic susceptibility: The Clinical and Laboratory Standards Institute-recommended Kirby Bauer Disc Diffusion method was used to test the bacterial strains for antibiotic susceptibility. Mueller Hinton agar was covered with commercially available antimicrobial discs containing amikacin, ceftazidime, cefotaxime, ciprofloxacin (5 µg), and levofloxacin.

After the plates were incubated, they were inspected, and the inhibitory zone diameters of each antimicrobial agent were measured. Based on the breakpoints for the corresponding antibiotic susceptibility, these diameters were reported as susceptible and resistant. MDR organisms are resistant to three or more antibiotic classes. The isolates underwent additional screening for ESBL and AmpC production, with phenotypic approaches serving as confirmation. The extended spectrum β lactamase (ESBL) was phenotypically detected using the double disk synergy test in accordance with CLSI and Amp C detection using the disc approximation test [6,7].

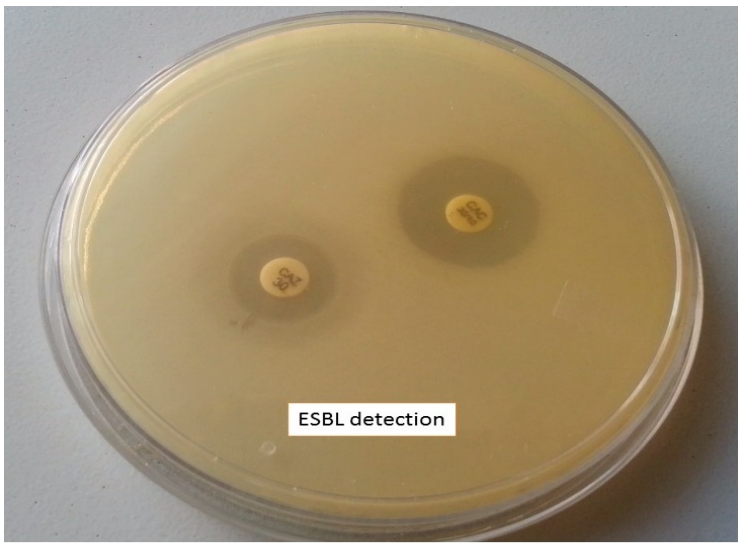

Figure 1: ESBL Detection: Using CAZ discs (ceftazidime), isolates were screened for ESBL. When a resistance pattern is seen, this is confirmed using the Double Disc Synergy method

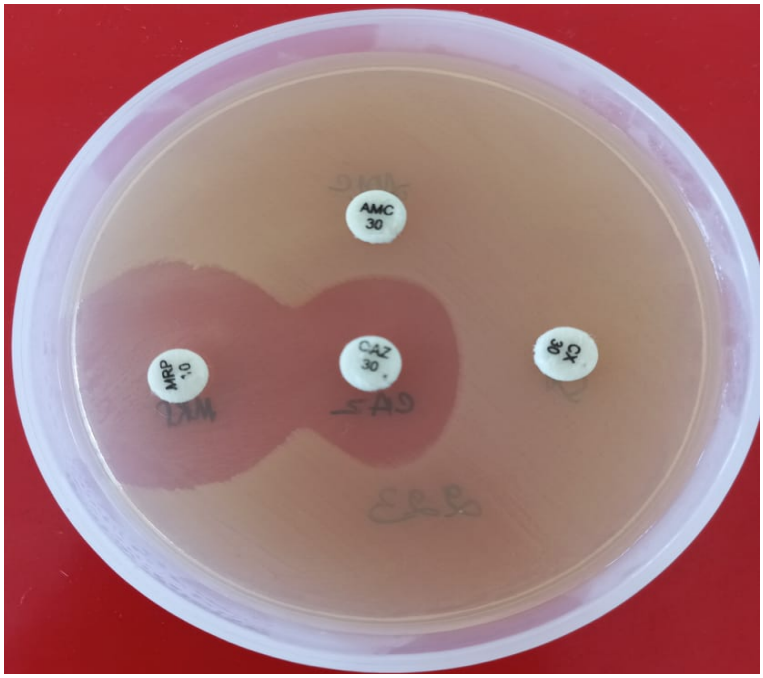

Figure 1: AmpC detection: Using a Cefoxitin disc, the isolates are additionally examined for the formation of AmpC, which is then verified by the Disc Approximation Test

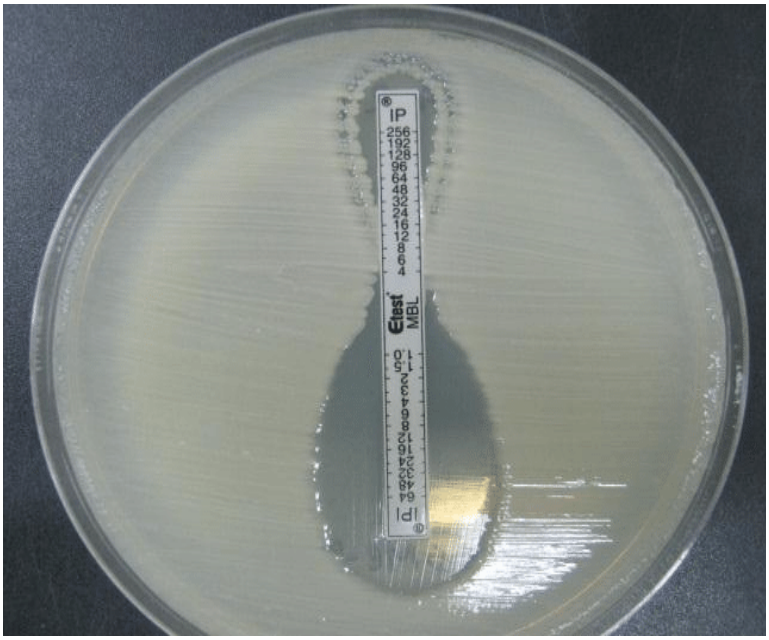

Figure 3: MBL Detection

Additionally, using the MBL E test method (HI MEDIA), the production of Metallo β lactamase (MBL) was examined in all the isolates resistant to carbapenem, with a ratio of MIC of imipenem to MIC of imipenem + ethylene diamine tetra acetic acid (EDTA) >8 [8].

Results:

Escherichia coli and K. pneumoniae, two members of the Enterobacteriaceae family, were isolated from every cultivated sample. Out of the 60 subjects, 100% had E. Coli, 80% had K. pneumoniae, and 20% had a combination of both E. Coli and K. pneumoniae. E. Coli was the most common type of flora, followed by Klebsiella pneumoniae. Similar outcomes were observed in a 2019 study conducted by Madhu et al., [9].

E. coli was the most frequently isolated bacterium in a related study conducted in Puducherry by Sherly et al., followed by Klebsiella [10]. Out of the 60 stool samples that were taken, women made up 71.6% (43/60) while men made up 28.3% (17/60).

The bulk of the stool samples were taken from female subjects. According to Figure 4 results of

the antimicrobial susceptibility assay using the disc diffusion method, 70% of gut bacteria were multidrug resistant (MDR), meaning they were resistant to various antimicrobial medications. The most common MDR bacterium among them was E. Coli (55%) followed by Klebsiella spp. (46%).Our findings seem consistent with other reports of drug resistant bacteria found in the guts of healthy youngsters [11, 12]. These two microbes have demonstrated resistance to multiple antibiotic classes. Cephalosporins exhibited the highest level of resistance (100%) followed by fluoroquinolones (50%) and aminoglycosides (43%).

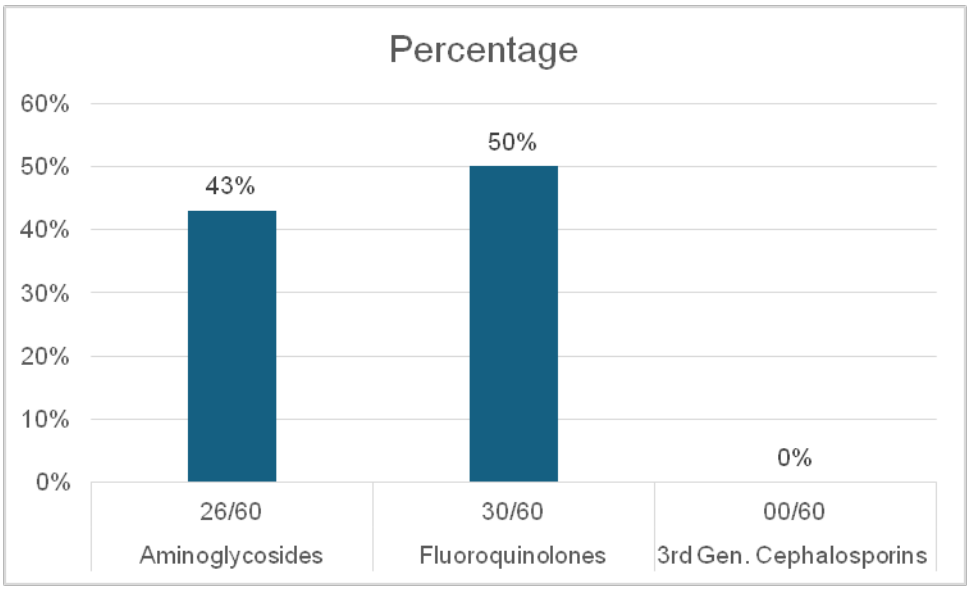

Figure 4: Antibiotic Sensitivity Pattern of the Isolates

Resistance of Cephalosporins (60.4%) and fluoroquinolones (41.5%) was observed In his study on Enterobacteriaceae In Healthy Gut Flora, Gupta et al. In a study by Kothari et al., extensive resistance to ampicillin (87%) and cephalosporins was observed in the gut colonization of healthy infants who were exclusively breastfed. [13] Since carbapenems and aminoglycosides are mostly used in hospital settings, there was comparatively less resistance seen in this study.

Table 1: Isolates Resistance Pattern against Various Antibiotic Classes

Class of Antibiotic	Number	Percentage
Cephalosporins + Aminoglycosides	14/60	23.33%
Aminoglycosides + Fluoroquinolones	NIL	-
Cephalosporins + Fluoroquinolones	14/60	23.33%
Cephalosporins + Aminoglycosides + Fluoroquinolones	20/60	33.33%
All sensitive	NIL	-

In the study E. Coli and Klebsiella isolates exhibited the highest rate of drug resistance to more than three antibiotic classes, including aminoglycosides, cephalosporins, and fluoroquinolones. According to the government of India's "scoping report on antimicrobial resistance in India (2017)," nearly half of all Pseudomonas aeruginosa isolates and over 70% of isolates of Escherichia coli, Klebsiella pneumoniae, and Acinetobacter baumannii were resistant to fluoroquinolones and third-generation cephalosporins among the Gram-negative bacteria [14].

Table 2: Percentage of Esbl, Ampc and Carbapenemase Producers

Type of Beta Lactamase	Number	Percentage
ESBL	17/60	28.57%
AmpC	09/60	14.28%
ESBL + AmpC	14/60	22.85%
Carbapenamase	10/60	17.14%

Further testing was done to determine whether isolates resistant to cefotaxime or ceftazidime produced ESBL and AmpC. The production of AmpC was 14.2% and ESBL was 28.5% overall.

AmpC and ESBL coproducers made up 22.8%.The production of Carbapenamase using the E test technique was 17%. A study by Kothari et al. revealed that 20.6, 19.9, and 11.2 percent of isolates had ESBL, AmpC, and coproduction of both [15]. A study from Pondicherry comparing hospitalized patients' concurrent ESBL E. Coli infection and gut colonization revealed a 21% faecal carriage rate. Beta-lactamases, also called penicillinase, are enzymes produced by bacteria that can break down β-lactam antibiotics, including cephamycin, carbapenems, and penicillin's (ertapenem).

The development of novel enzymes like TEM-, SHV-, and OXA-type βlactamases is largely responsible for the establishment of βlactam resistance in Enterobacteriaceae. Given that Enterobacteriaceae members are known to produce both community-acquired infections and hospital acquired illnesses, this could be a risk factor that favours the community spread [16]. E. Coli and K. pneumoniae belong to the Enterobacteriaceae family and are known to cause UTIs that, if left untreated, might worsen. The current investigation revealed that ESBL and AmpC co-occurred in 28.5% of isolates, which is greater than the 5.7% found in the Chanu et al. study [17]. In comparison to the research conducted by Mirza et al., our findings for MBL production was found to be somewhat higher (17%)[18].

Discussion:

The information from this study sheds light on the frequency of multidrug-resistant (MDR) bacterial species belonging to the Enterobacteriaceae family in the guts of healthy people. The most resistance to cephalosporins (60.4%) was observed, with fluoroquinolones (41.5%) coming in second. As India is reportedly the world's largest consumer of antibiotics for human use, one plausible explanation for this could be the irrational usage of these drugs in the community [19]. Additional factors may include patients' self-prescription practices and healthcare professionals' irrational prescription of medications, particularly fixed-drug combinations [20].

Globally, β-lactam antibiotics are the most often administered class of antibiotics. There is evidence of the widespread distribution and plasmid mediated transmission of ESBLs, with ESBL infections linked to communities. Under the right circumstances, ESBL producing bacteria in the gut can cross the intestinal barrier and manifest as bacteraemia. These organisms operate like opportunistic pathogens. ESBL producing E. coli has been linked to community onset urinary tract infections in recent reports [21]. The shifting patterns in commensal E. coli resistance to widely used antibiotics in the gut, as observed by multiple researches on colonization in the Indian population, might be an indication of shifting patterns in antibiotic prescription over time [22–24].

In a 2011 study from Uttar Pradesh, the prevalence rates of AmpC (AmpC disk test using TrisEDTA) and ESBL (double disc synergy test) in 396 community dwelling healthy persons were 51% and 42%, respectively [25]. The resistance of AmpC β-lactamases producers to cefoxitin, β-lactam and β-lactam inhibitor combinations, further reduces the range of available treatment choices, making them a serious cause for concern. Since ESBL and/or AmpC-producing bacteria are treated with carbapenems, the coexistence of these enzymes can be dangerous for community acquired pathogens because the minimum inhibitory concentration (MIC) of these strains is ten times greater for different carbapenems [26].

The possibility of several resistance enzyme genes being transferred horizontally within a single strain is suggested by the co-production of various β lactamases in our investigation. This underlines once again how crucial it is to have ongoing monitoring, particularly for MDR Enterobacteriaceae in both hospital and community settings, to provide prompt and appropriate therapy. Therefore, to prevent therapeutic failure and exacerbate the current state of antimicrobial resistance, such MDR organisms should always be tested for and treated with the appropriate antibiotics whenever they are isolated. The high MDR rate seen in this sparsely populated semiurban area indicates that threat is most in India's more populous cities. The finding of resistant isolates in this population's gut flora, is alarming.

The study shows that organisms with resistance mechanisms, like Carbapenemases and ESBL are proliferating in the community. When initiating empiric therapy for a significant bacterial infection in a patient, it is important to anticipate these resistance mechanisms. Health care professionals and laypeople in these locations need to be made aware of antibiotic recommendations and awareness. Regular testing for common resistance mechanisms like ESBLs and Carbapenemases, particularly in patients admitted to intensive care units (ICUs), may aid in understanding the dynamics of antibiotic resistance and aid in preventing its emergence.

One of the study's main limitations was that stool samples were taken at random from community members without screening the entire population. It would have been intriguing to assess the current level of antibiotic resistance in the food items this population consumes and compare it to the microbiota in their faeces. To determine the actual

number of gut resistant colonizers in the population, active surveillance is crucial.

Conclusion:

It is concerning to find commensal organisms with resistance genes. There is a chance that this will spread and might manifest them when antibiotic pressure is present. The way that community acquired infections will be negatively impacted by the results of gene dissemination. To determine the actual prevalence of MDR in the community, more research involving environmental sampling and a correlation of local prescription patterns must be conducted. Continuous surveillance should be implemented in order to identify resistant isolates. It is important to guarantee that rules pertaining to the purchase of antibiotics, prescription of drugs, and patient compliance are strictly enforced. The spread of resistant bacteria may be stopped by using clean, safe drinking water and good hygiene and also by raising awareness of the community's usage and abuse of antibiotics.

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