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Original Article

Isolate and Identify *Enterobacter* Species from Different Clinical Samples and Determine Sensitivity Patterns

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ABSTRACT

Enterobacter, a genus in the Enterobacteriaceae family, includes 22 recognised species, some of which are linked to health-related infections. Though not all cause diseases, Enterobacter is implicated in hospital-acquired and, to a lesser extent, community-acquired infections like urinary tract infections and soft tissue infections. The aim of the study was to isolate and identify Enterobacter species from different clinical samples and to determine antimicrobial sensitivity patterns. This cross-sectional study conducted in a well-equipped private laboratory in the Sylhet region from January to December 2022 aimed to comprehensively investigate the isolation, identification, and sensitivity patterns of Enterobacter species across various clinical samples. A total of 400 cases with clinical symptoms suggestive of infections were enrolled in the study by purposive sampling. In this study encompassing 400 clinical samples, 71 (17.8%) cases yielded positive cultures of Enterobacter species, with urine samples exhibiting the highest culture positivity rate at 22.2%, followed by wound swabs at 15.5%, and blood at 5%. The predominant species identified were E. Cloacae (76.1%) and E. aerogenes (23.9%). Antibiotic resistance patterns varied, with amikacin and levofloxacin showed the highest sensitivity at 94.4% and 91.6%, respectively, while cefixime and meropenem demonstrated the highest resistance at 69% and 66.2%, respectively. The study highlighted that Enterobacter exhibited diverse responses to different antibiotics, with the highest sensitivity observed for amikacin and levofloxacin, while cefixime and meropenem had the highest resistance. Higher resistence to multiple antibiotics posing challenges in clinical management.

Keywords: *Enterobacter, Clinical samples, Sensitivity patterns.*

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INTRODUCTION

Enterobacter, a genus within the *Enterobacteriaceae* family, is primarily associated with healthcare-related infections. *Enterobacter* were proposed as a genus in 1960 by Hormaeche and Edwards based on the division

of the former genus *Aerobacter*. Presently, there are 22 recognised species of *Enterobacter*. Nevertheless, not all of these species are acknowledged as disease-causing agents. *Enterobacter* species play a role in numerous nosocomial infections and, to a lesser extent, community-acquired infections. These encompass conditions such as urinary tract infections (UTIs), soft tissue infections, osteomyelitis, endocarditis, and various others. *Enterobacter* has become increasingly resistant to many previously effective antibiotics. The genus *Enterobacter* includes facultative anaerobic gram-negative bacilli that are 12 nm long, are motile by means of peritrichous flagella, and belong to the family *Enterobacteriaceae*. The genus *Enterobacter* is associated with a variety of environmental habitats. These bacteria are recovered from soil and water and are endophytic, or considered phytopathogens for various plant species[1]. Few studies have investigated the potential virulence associated with the genus *Enterobacter* in human infections. The lack of significant research in this area is probably a reflection of the predominant role of these organisms as nosocomial pathogens[2].

Enterobacter spp., including *E. aerogenes* and *E. cloacae* easily acquires numerous genetic mobile elements containing resistance and virulence genes which strongly contribute to the increased pathogenicity of the bacteria. These organisms seem to have an innate resistance to older anti-microbial agents and the propensity to rapidly develop resistance to newer antimicrobial agents[2]. Treatment of infections with *Enterobacter* spp. is difficult, and broad resistance to third generation cephalosporins, penicillin, and quinolones is an increasing problem[3]. Additionally, these microbes have the chromosomally encoded qnr gene, which likely provides a genetic background that is favourable for the establishment of additional quinolone-targeted mutations and the eventual development of fluoroquinolone resistance[4]. Aminoglycoside modifying genes that were involved in aminoglycoside resistance are aac (3)-IIa, aac (6')-Ib, and ant (2")-Ia, genes confer resistance to tobramycin, gentamicin, and amikacin[4]. For gram-negative pathogens, including *Enterobacter* species, colistin is often recommended as a last-line therapeutic option[5]. *Enterobacter* spp. is resistant to last-resort antibiotics, such as tigecycline or colistin due to impaired uptake and enhanced pump-out has been reported[6]. The presence of transferable mechanisms of colistin resistance has also been reported in *Enterobacter* spp. from hospital environments[7].

Epidemiology: By the 1970s, it was established that *Enterobacter* spp. could be nosocomial pathogens, although they were much less commonly encountered than *Escherichia coli* and *Klebsiella* strains. However, the importance of *Enterobacter* spp. as a nosocomial pathogen was highlighted in the National Nosocomial Infections Surveillance System (NNIS) data. Although community-acquired infections with *Enterobacter* spp. do occur, the majority of infections with this organism are nosocomial. Patients at increased risk of acquiring an *Enterobacter* infection include those with a prolonged hospital stay, especially if a portion of it is spent in an ICU. *E. cloacae* has been repeatedly reported as a nosocomial pathogen in neonatal units, and several outbreaks of infection have been reported. Epidemiology has helped develop methodologies used in clinical research, public health studies, and, to a lesser extent, basic research in the biological sciences[8].

Natural habitat: Members of the genus *Enterobacter* are environmental organisms and opportunistic pathogens of plants and humans. *E. aerogenes* is frequently isolated from respiratory, urinary, blood, or gastrointestinal tract specimens. *E. cloacae* are ubiquitous in terrestrial and aquatic environments (Water, sewage, soil, and food). The species occurs as commensal microflora in the intestinal tracts of humans and animals and is also a pathogen in plants and insects. This diversity of habitats is mirrored by the genetic variety of *E. cloacae*[9].

Laboratory diagnosis: This organism is easy to isolate from clinical specimens and biochemical tests readily separate it from other members of the *Enterobacteriaceae* family. They produce pink colonies in MacConkey's agar media (Oxoid, UK) after streaking and are incubated for 24 hours in an incubator for the isolation of the probable pathogenic bacteria. Bacterial colonies on the media plates were later gram stained and identified by colonial morphology. Some strains with a K antigen possess a capsule. Colonies of *Enterobacter* strains may be slightly mucoid. On biochemical test, acidic (Yellow) slant and butt with gas production but no H_2S in TSI agar. *E. aerogenes* is urease negative, whereas *E. cloacae* showed variable results. *E. aerogenes* are lysine decarboxylase positive, and *E. cloacae* are negative for this test. They are catalase-positive and oxidase negative[10].

The objectives of the study were to isolate and identify *Enterobacter* species from different clinical samples and to determine antimicrobial sensitivity patterns.

METHODS AND MATERIALS

This cross-sectional study was conducted within a well-equipped private laboratory Popular Medical Center Ltd., Sylhet. A total of 400 samples were included by purposive sampling, of which 71 yielded positive cultures. The study was conducted over the period of

January 2022 to December 2022. The study included cases that exhibited clinical symptoms suggestive of infections. Aseptically collected clinical samples, such as wound swabs, urine samples, and blood specimens, were included in the study. Duplicate or redundant samples from the same individual and samples deemed inadequate in terms of volume or quality for meaningful testing were excluded. The specimens, after collection, were cultured in Mac Conkey's agar media and were incubated for 24 hours in an incubator. After that, different *Enterobecter* species were isolated by various biochemical tests. The isolated *Enterobacter* species underwent thorough antimicrobial sensitivity testing by the Kirby-Bauer disc diffusion method according to National Committee for Clinical and Laboratory Standards Criteria (NCCLS)[11]. Arrays of clinically significant antimicrobial agents were introduced to the isolates, encompassing antibiotics commonly deployed in clinical practice. The zones of inhibition, a crucial metric, were meticulously measured and subsequently interpreted in alignment with established guidelines as recommended by NCCLS, thus revealing the sensitivity patterns of the isolates. A written consent was obtained from the head of the department of pathology and microbiology, Popular Medical Center Ltd., Sylhet. Data were collected in a predesigned questionnaire. The collected data were analyzed manually and presented as frequency and percentage.

RESULTS

A total of 400 samples were included in the study. Among them urine, wound swabs, and blood, comprising 230, 110, and 60 specimens, respectively. Out of 400 samples, 71 (17.8%) yielded positive cultures. Among them, 51 (22.2%) of the urine, 17 (15.5%) of the wound swab, and 3 (5%) of the blood samples yielded growth of *Enterobacter* in culture (Figure-I).

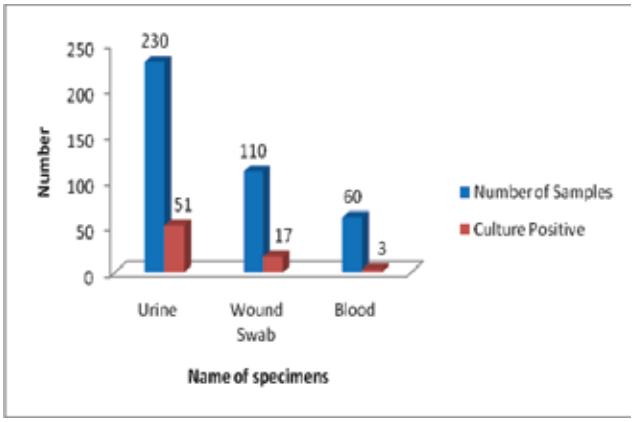

Figure-1: *Culture Positivity from various clinical samples, N=400*

Figure-2: *Presence of Enterobacter in Mac Conkey Agar media*

Figure-2 shows the presence of *Enterobacter* in Mac Conkey Agar media and table-I shows biochemical characteristics for the identification of species of the genus *Enterobacter.*

Figure-3: *Biochemical test for diagnosis of Enterobacter*

Table-I: *Biochemical characteristics for the identification of species of the genus Enterobacter*

Species	MIU medium			TSI medium				Citrate	LDC	Oxidase
	Motility	Indole	Urea	Butt	Slant	H_2S	Gas			
Enterobacter aerogenes	+	-	-	Y	Y	-	++	+	+	-
Enterobacter cloacae	+	-	D	Y	Y	-	++	+	-	-

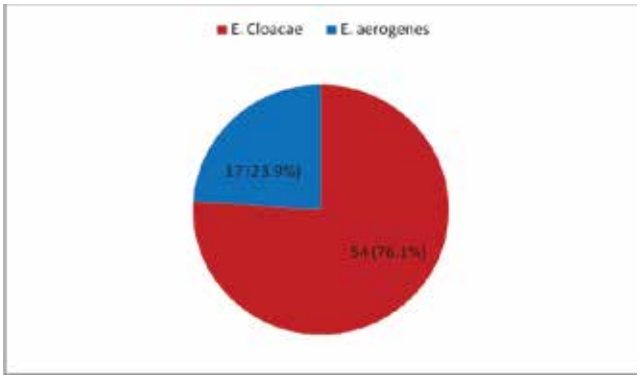

Figure-4: *Different Enterobacter spp. identified by culture, biochemical test, N=71*

Figure-4 presents the outcomes of culture and biochemical tests conducted on 71 cases to identify different *Enterobacter* species. The figure highlights two specific species, *E. Cloacae*, and *E. aerogenes*, indicating their respective counts of culture-positive cases, with *E. Cloacae* comprising the majority at 76.1%, and *E. aerogenes* accounting for the remaining 23.9%.

occurrence of 13.5% for *E. cloacae* across all samples. For *E. aerogenes*, 4.7% of urine samples, 5.4% of wound swab samples, and no instances in blood samples were recorded, amounting to a total occurrence of 4.3% across all samples.

Table-III provides a comprehensive overview of the antibiotic resistance patterns observed among isolated *Enterobacter* spp. from a total of 71 cases. The table shows the number and percentage of instances exhibiting sensitivity and resistance to each antimicrobial medication. Notably, *Enterobacter* exhibited diverse responses to different antibiotics, with the highest sensitivity observed for amikacin at 94.4%, while cefixime had the highest resistance rate at 69%.

DISCUSSION

Due to the challenges of treating multidrug resistant (MDR) microorganisms and the exponential rise in MDR over the past ten years, multidrug resistance is the most significant issue in medical practice[12]. The emergence of multidrug resistant *Enterobacter* spp. isolates have had a negative impact on the clinical outcome of infected patients and increasing mortality rates[6].

Enterobacter species have frequently caused

Table-II: *Distribution of E. cloacae and E. aerogenes isolated from different samples, N=400*

Organism	Urine N=230 n (%)	Wound Swab N=110 n (%)	Bloo d N=60 n (%)	Total N=400 n (%)
E. cloacae	40 (17.3)	11 (10)	3 (5)	54 (13.5)
E. aerogenes	11 (4.7)	6 (5.4)	0 (0)	17 (4.3)
Total	51 (22.17)	17 (20.9)	3 (5)	71 (17.8)

Table-II illustrates the distribution of *E. cloacae* and *E. aerogenes* bacteria across distinct clinical samples from a total of 400 cases. Among these, 17.3% of urine samples, 10% of wound swab samples, and 5% of blood samples contained *E.cloacae,* resulting in an overall

Table-III: *Antibiotic sensitivity pattern of isolated Enterobacter spp., N=71*

Antimicrobial drugs	**Sensitivity pattern**	***Enterobacter, n***	**Percentage (%)**
Amoxiclav	S	44	62
	R	27	38
Piperaciline Tazobactam	S	36	50.7
	R	35	49.3
Cefuroxime	S	56	78.9
	R	15	21.1
Ceftriaxone	S	61	85.9
	R	10	14.1
Ceftazidime	S	57	80.3
	R	14	19.7
Cefepime	S	37	52.1
	R	34	47.9
Cefixime	S	22	31
	R	49	69
Meropenem	S	24	33.8
	R	47	66.2
Amikacin	S	67	94.4
	R	04	5.6
Ciprofloxacin	S	61	85.9
	R	10	14.1
Levofloxacin	S	65	91.5
	R	06	8.5
Cotrimoxazole	S	53	74.6
	R	18	25.4
Netilmycin	S	42	59.2
	R	29	40.8

nosocomial infections in recent decades. In our study, a total of 400 samples were included of which 71 yielded positive cultures. These findings are in agreement with a recent study in DMCH by Haque A[13] reported that 10.53% samples (Urine, woundswab, ETA, Blood) were culture positive, respectively. Patel et al.[2] reported in a review article that in India *Enterobacter* accounts for 5-11% of all nosocomially acquired blood, wound, respiratory tract infection. These findings are nearly close to the present findings.

Among the different samples in our study, urine exhibited the highest culture positivity rate (22.2%), followed by wound swabs (15.5%), and blood (5%). Overall, out of the total samples analysed, 71 (17.8%) cases were culture-positive. This finding is similar to the findings reported in a article by Davin-Regli et al[1]. In the present study, among 71 isolated *Enterobacter* species, 54 (76.1%) were identified as *Enterobacter cloacae* and 17 (23.9%) were identified as *Enterobacter aerogenes* by biochemical tests. These findings were similar to the recent study in DMCH by Haque A[13] who found that 24 (82.76%) were *E. cloacae* and 5 (17.24%) were *E. aerogenes*. Moreover, a study in India by Sujatha et al.[14] who reported that 77.94% were *E. cloacae* and 22.05% were *E. aerogenes*. This similarity may be attributed to the fact that these two studies were conducted in the same geographic area. In the present study, 14 commonly used antibiotics were used on isolated *Enterobacter* species to observe the antimicrobial sensitivity and resistance pattern by disc diffusion method. Among the antibiotics used, *Enterobacter* spp. was highly resistant to cefixime and meropenum. These findings are correlated with the study of Adhikari et al[15].

In our study, cefuroxime was 78.9%, ceftazidime was 80.3%, and ceftriaxone was 85.9% sensitive to *Enterobacter species*. In a study in Nepal found that ceftazidime and ceftriaxone were 55.8%, and 58.8% sensitive, respectively[16]. These findings were nearly similar to our study. In our study, we found *Enterobacter* spp. were 94.4% sensitive and 5.6% resistant to amikacin, which was in accordance with a study conducted by Ghimire et al[16]. In contrast to present findings in Iran, Mortazavi et al.[17] reported 48.6% *Enterobacter* resistant to amikacin which is higher than the present findings. The reason behind the higher resistance rate in that study might be due to a significant irrational use of antibiotics.

In a study Ozcan et al.[18] found that the sensitivity of ciprofloxacin was 81%, which is nearly similar to our study. We found the sensitivity rate of ciprofloxacin was 85.9%. The rise of multidrug-resistant organisms poses a significant global challenge for clinicians and public health. The emergence of multidrug resistance in *Enter obacter* spp. creates a pressing therapeutic predicament, limiting effective options for treating severe infections.

LIMITATIONS

While this study represents a comprehensive endeavor, it is essential to acknowledge that the results may be influenced by the single private laboratory setting, potentially limiting their generalizability to broader populations. The relatively contained study period of a single calendar year could potentially restrict the observation of longer-term trends.

CONCLUSION

In conclusion, this year-long cross-sectional study in the Sylhet region provided valuable insights into *Enterobacter* species, their prevalence, and antimicrobial sensitivity patterns. The study highlighted that *Enterobacter* exhibited diverse responses to different antibiotics, with the highest sensitivity observed for amikacin and levofloxacin, while cefixime and meropenem had the highest resistance. The study highlighted the increasing resistance of *Enterobacter* to multiple antibiotics, posing challenges in clinical management. The meticulous data analysis, conducted in a well-equipped private laboratory, emphasises the importance of ongoing surveillance to address the evolving threat of *Enterobacter* infections and guide effective treatment strategies in clinical settings.

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