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Post-Transplant Fecal Carriage of Antibiotic Resistant and β-Lactamases-Producing Enterobacteriales among Renal Transplant Recipients

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ABSTRACT

Background: The intestinal colonization and transmission of antibiotic-resistant Enterobacteriales to renal transplant recipients may pose a threat to them because they are profoundly immunocompromised and vulnerable to infection. Hence, it is crucial to identify these antibiotic-resistant fecal Enterobacteriales harboring high-risk populations. The objective of this study was to determine antibiotic resistance as well as β-lactamases production in fecal Enterobacteriales among renal transplant recipients.

Methods: The stool samples, one collected from each transplant recipient, were processed for isolation and identification of Enterobacteriales and were tested for their antibiotic susceptibility, extended-spectrum β-lactamase, and metallo-β-lactamase production by standard methods.

Results: A total of 103 Enterobacteriales comprising of Escherichia coli (86.4%), Klebsiella species (11.7%), and Citrobacter species (1.9%) were isolated and more than 60% of the E. coli were found resistant to ceftazidime and ciprofloxacin and around half of the Klebsiella species were resistant to ceftazidime and fluroquinolones. The extended-spectrum β-lactamase production was seen in 3.4% and 8.3% and metallo-β-lactamase production in 24.7% and 33.3% of E. coli and Klebsiella species, respectively. The high proportion of β-lactamase-producers were resistant to piperacillin-tazobactam, meropenem, gentamicin, and amikacin than β-lactamases non-producers.

Conclusion: Since the antibiotic resistance is higher in fecal Enterobacteriales, each renal transplant recipient should be screened for these highly resistant intestinal colonizers after transplantation in order to prevent infections and to reduce the rate of transplant failure due to infections.

Keywords: Antibiotic resistance; β-lactamases; enterobacteriales; fecal carriage; renal transplantation.

INTRODUCTION

Pathogenic and commensal bacteria have developed resistance to several antibiotics.[1,2] The extended-spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs)-producing *Escherichia coli (E. coli)* and *Klebsiella* species have emerged worldwide as a frequent cause of infections.[3-5] Infections due to ESBL-producing Enterobacteriales (ESBL-E) and carbapenem-resistant Enterobacteriales (CRE), have been on the rise in infecting solid organ transplant (SOT) recipients and are associated with increased risks for infection.[6-8]

The colonization of antibiotic-resistant Enterobacteriales to renal transplant recipients may pose a threat to them because they are vulnerable to infections caused by these intestinal flora.[4] Our concern is that kidney transplants are under immunosuppressive medications and their fecal flora may contain a variety of Enterobacteriales that are antibiotic-resistant and β-lactamase producers.

The objective of this study was to determine antibiotic

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resistance as well as ESBL and MBL production in fecal Enterobacteriales among recipients after renal transplantation.

METHODS

This cross-sectional descriptive study was conducted between the period of June 2018 to May 2019 among renal transplant recipients attending Shahid Dharmabhakta National transplant Center (SDNTC)/ Human Organ Transplant Center (HOTC), Nepal after transplantation. This study was approved by Ethical Review Board of Nepal Health Research Council, Kathmandu, Nepal (Reference No.: 2998/2019). Before enrollment in the study, informed consent was obtained from each participant. In this study, any renal transplant recipients visiting the center for medical consultation regardless of their transplant duration and provided consent to participate in the study were included. The participants were provided with a sterile wide-mouthed screw-capped container and were clearly instructed on how to collect the stool samples.

One stool sample was collected from each participant and was immediately transported to the microbiology laboratory and processed for the isolation and identification of Enterobacteriales using standard microbiological procedures.[9] The stool samples were inoculated onto a single MacConkey agar plate, which was purchased from HiMedia Laboratories, India, and incubated overnight aerobically at 37°C. The colony characteristics, Gram's staining of culture smears, motility testing, and a series of biochemical tests were used to identify each morphological type of bacterial colonies grown on the plate.[1,9] The Enterobacteriales isolates recovered from the stool were tested for their antibiotic susceptibility profiles by Kirby-Bauer disk diffusion method following the instructions provided in the Performance Standards for Antimicrobial Susceptibility Testing (M100 Document, 2017) by Clinical and Laboratory Standards Institute (CLSI). The CLSI recommended quality control strain *E. coli* ATCC 25922 was also tested in every set of experiments for validity of the antibiotic susceptibility test.[10]

The screening and confirmation of ESBL production in Enterobacteriales isolates were carried out in accordance with the CLSI guidelines (M100 Document, 2017). The isolates with an inhibition zone of ≤22 mm for ceftazidime (30 µg) in the antibiotic susceptibility test were considered as probable ESBL producers. The ESBL production in Enterobacteriales isolates was confirmed by using a combination disk test with ceftazidime (30 µg) alone and its combination with a ß-lactamase inhibitor [ceftazidime-clavulanate (30 µg/10 µg)] according to CLSI guideline. For the quality control of ESBL detection test, *K. pneumoniae* ATCC 700603 strain was used as a positive control and *E. coli* ATCC 25922 strain as a negative control.[5,10] The Enterobacteriales isolates that showed reduced susceptibility (zone of inhibition size <23 mm) to either imipenem (10 µg) or meropenem (10 µg) were selected for testing MBL production by the combination disk method. For the quality control of MBL detection test, *Pseudomonas aeruginosa* PA 105663 and *Pseudomonas aeruginosa* ATCC 27853 were tested as positive and negative controls, respectively.[11]

The data generated during the study were processed and analyzed using the Statistical Package for the Social Sciences (SPSS) version 16.0 (IBM, Armonk, NY, USA) and Microsoft Excel 2007, and the results were interpreted according to frequency distribution and percentage. The Pearson's χ^2 test was used to compare variables, and the data with a p-value <0.05 (95% CI) were considered significant.

RESULTS

A total of 103 renal transplant recipients participated during the study period, with 88 males and 15 females forming a male to female ratio of 5.87. The participants' ages ranged from 14 to 64 years (mean: 33.79; SD±9.872), the majority (67.0%) being between the ages of 21 years and 40 years with only two (1.9%) being over the age of 60 years. Of the total participants, 71.8% received renal transplant within the last six months at the time of enrollment in the study, 58.3% had a history of hospitalization for less than a week following transplantation, 26.2% had a history of multiple post-transplant hospital stays, 69.9% had recently used antibiotics and 59.2% had taken antibiotics intravenously after transplantation (Table 1).

A single stool sample collected from each participant was cultured for isolation and identification of Enterobacteriales, yielding an equal number (N=103) of Enterobacteriales isolates (one bacterial isolate from each sample). *E. coli* (86.4%) represented the most common bacterial isolates followed by *Klebsiella* species (11.7%; *K. pneumoniae*=7 and *K. oxytoca*=5) and *Citrobacter* species (1.9%). Out of the total number of *E. coli* isolates (n=89), 71.9% were isolated from participants who had undergone renal transplantation within six months. Similarly, out of total *Klebsiella* species (n=12), 66.6% of isolates and all *Citrobacter* species were recovered from participants who had

undergone renal transplantation within six months (Table 2).

E. coli isolates exhibited higher levels of resistance towards penicillin, cephalosporin, and fluoroquinolones. More than 80% of the *E. coli* isolates were resistant to ampicillin and amoxycillin-clavulanate, while more than 60% were resistant to ceftazidime, ciprofloxacin and ofloxacin, and these isolates were less resistant to piperacillin-tazobactam, carbapenems and aminoglycosides (Figure 1). Similarly, out of total *Klebsiella* species, all isolates were resistant to amoxycillin-clavulanate, and around half of the isolates were resistant to ceftazidime and fluroquinolones and these isolates also showed a lower level of resistance towards piperacillin-tazobactam, carbapenems and aminoglycosides (Figure 2). There were only two *Citrobacter* species isolated, and both were resistant to amoxycillin-clavulanate while both isolates were also susceptible to imipenem, meropenem, gentamicin, amikacin, and tobramycin.

This study also attempted to determine the antibiotic resistance rate of Enterobacteriales isolates in relation to the duration of renal transplantation. Enterobacteriales (*E. coli, Klebsiella* species, and *Citrobacter* species) isolated from recipients who had underwent renal transplantation within six months exhibited higher levels of resistance to all tested antibiotics than those isolated from recipients who underwent renal transplantation before six to 12 months and before more than 12 months ago (Figure 3).

Out of total 103 Enterobacteriales isolates, 30.1% were found ß-lactamase (ESBL or MBL) producers, with 4.9% being ESBL producers and 25.2% being MBL producers. The ESBL production was seen in 3.4% of total *E. coli* isolates, while MBL in 24.7% isolates. Similarly, 8.3% of *Klebsiella* species were tested positive for ESBL and 33.3% tested positive for MBL. Out of the two *Citrobacter* species, only one isolate produced ESBL, and none of the isolates produced MBL.

There was a substantial difference between the susceptibility to the tested antibiotics in ß-lactamase (ESBL or MBL) producing and non-producing isolates when comparing their antibiotic susceptibility profiles. Notably, the ß-lactamase producers were found to be significantly more resistant than the ß-lactamase non-producers to piperacillin-tazobactam, imipenem, meropenem, gentamicin, amikacin, and tobramycin (Table 3).

Table 1. Characteristics of renal transplant recipients enrolled in the study (N=103).

Characteristics		Number	Percentage
Sex	Male	88	85.4
	Female	15	14.6
Age group	≤20 Years	6	5.8
	21-40 Years	69	67.0
	41-60 Years	26	25.2
	>60 Years	2	1.9
Transplant duration	<6 months	74	71.8
	6-12 months	21	20.4
	>12 months	8	7.8
History/ duration of hospital stay	No history of hospital stay	18	17.5
	Stay less than a week at hospital	60	58.3
	Stay more than a week at hospital	25	24.3
Multiple stay at hospital	Yes	27	26.2
	No	76	73.8
Antibiotics used recently	Yes	72	69.9
	No	31	30.1
Antibiotics used intravenously	Yes	61	59.2
	No	42	40.8

Table 2. Transplant duration wise distribution of fecal Enterobacteriales isolated from renal transplant recipients.

Fecal Enterobacteriales isolates	Number of bacterial isolates (%)		
	<6 months	6-12 months	>12 months
E. coli (n=89)	64 (71.9)	19 (21.3)	6 (6.8)
Klebsiella species *(n=12)*	8 (66.6)	2 (16.7)	2 (16.7)
Citrobacter species *(n=02)*	2 (100)	0 (0)	0 (0)
Total (N=103)	74 (71.8)	21 (20.4)	8 (7.8)

Table 3. Antibiotic susceptibility profile of ß-lactamases (ESBL or MBL)-producing and non-producing fecal Enterobacteriales.

Antibiotics	AST results	ß-Lactamases producers (n=31)	ß-Lactamases non-producers (n=72)	p-value
Amoxicillin-clavulanate	Resistant (%)	31 (100)	55 (76.4)	0.003
	Susceptible (%)	0 (0)	17 (23.6)	
Piperacillin-tazobactam	Resistant (%)	27 (87.1)	12 (16.7)	<0.001
	Susceptible (%)	4 (12.9)	60 (83.3)	
Ceftazidime	Resistant (%)	31 (100)	31 (43.1)	<0.001
	Susceptible (%)	0 (0)	41 (56.9)	
Imipenem	Resistant (%)	27 (87.1)	11 (15.3)	<0.001
	Susceptible (%)	4 (12.9)	61 (84.7)	
Meropenem	Resistant (%)	26 (83.9)	15 (20.8)	<0.001
	Susceptible (%)	5 (16.1)	57 (79.2)	
Ciprofloxacin	Resistant (%)	28 (90.3)	36 (50.0)	<0.001
	Susceptible (%)	3 (9.7)	36 (50.0)	
Ofloxacin	Resistant (%)	27 (87.1)	39 (54.2)	0.001
	Susceptible (%)	4 (12.9)	33 (45.8)	
Gentamicin	Resistant (%)	22 (71.0)	16 (22.2)	<0.001
	Susceptible (%)	9 (29.0)	56 (77.8)	
Amikacin	Resistant (%)	20 (64.5)	11 (15.3)	<0.001
	Susceptible (%)	11 (35.5)	61 (84.7)	
Tobramycin	Resistant (%)	21 (67.7)	11 (15.3)	<0.001
	Susceptible (%)	10 (32.3)	61 (84.7)	

AST, antibiotic susceptibility test.

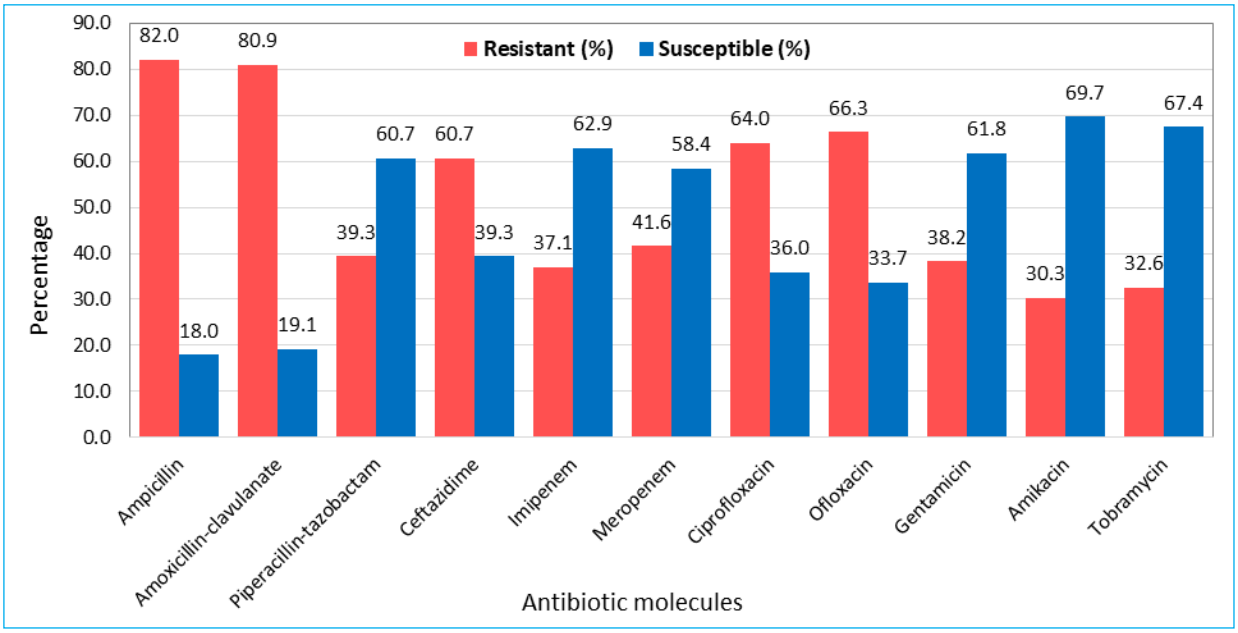

Figure 1. Antibiotic susceptibility profile of fecal Escherichia coli (n=89) isolated from renal transplant recipients.

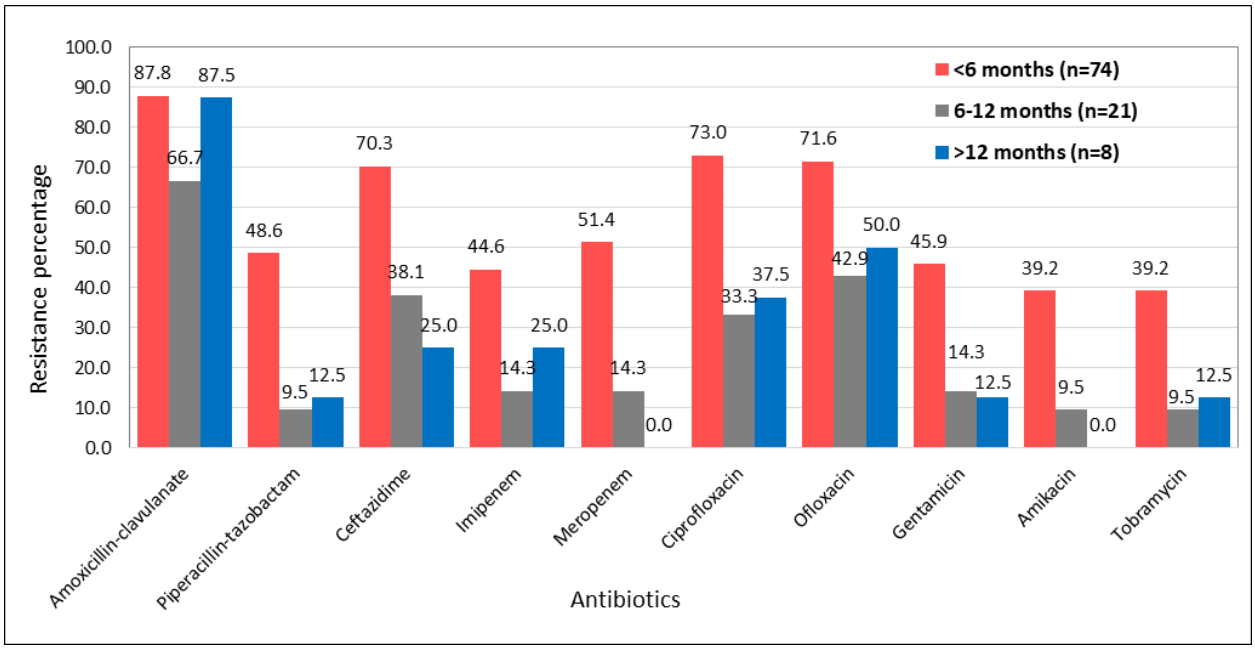

Figure 2. Antibiotic susceptibility profile of fecal Klebsiella species (n=12) isolated from renal transplant recipients.

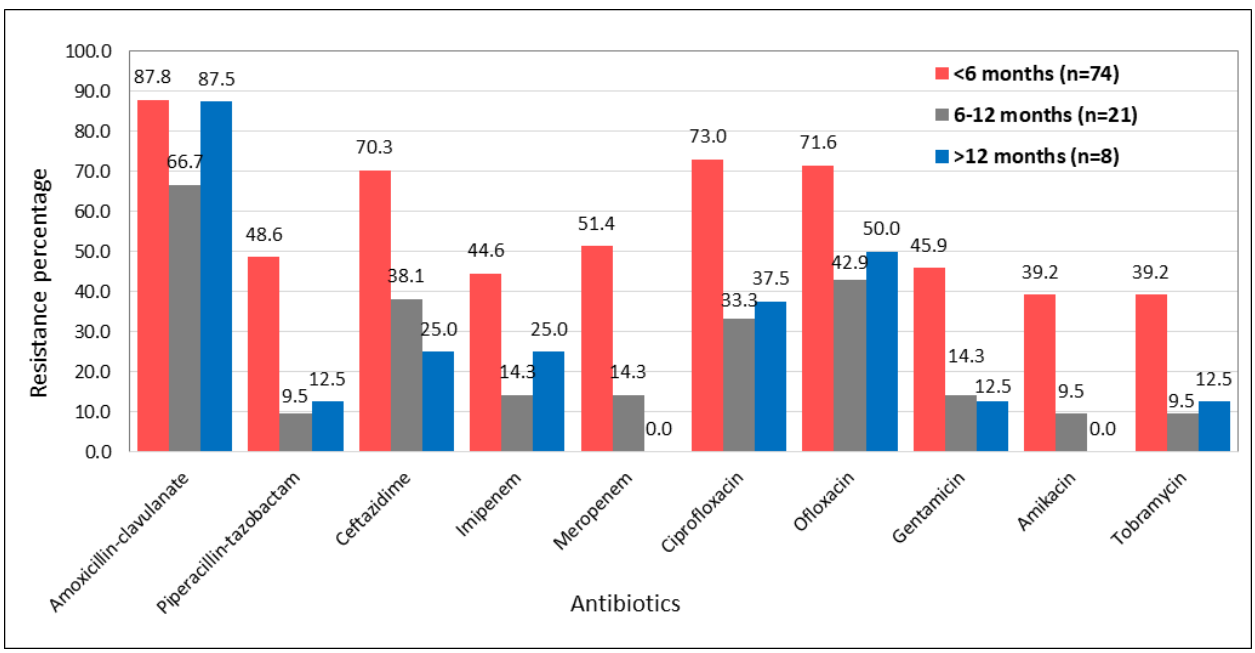

Figure 3. Transplant duration wise resistance pattern of fecal Enterobacteriales isolated from renal transplant recipients.

DISCUSSION

Antibiotic resistance has now been spread to a variety of pathogenic as well as commensal bacterial species and has been identified as a significant cause of death worldwide that is expected to worsen over the next few decades.[1,3,12] The SOT recipients are exposed to several risk factors for colonization and infection with antibiotic resistant bacteria, most of them requiring central venous lines, urinary catheterization, abdominal drainage or mechanical ventilation, which are all sources of bacterial infections.[13,14] Renal transplant recipients after surgical procedure remain in the hospital for a period of time and undergo antibiotic prophylaxis as well as immunosuppressive drug therapy, they are more prone to several endogenous infections.[15] Concerning these issues, we have determined antibiotic resistance and ß-lactamases production in fecal Enterobacteriales isolated from renal transplant recipients. Although members of the Enterobacteriales generally reside in the gut without harm, they are capable of producing a variety of infections, the most frequent is a urinary tract infection and the risk of infection is higher in transplant recipients.[16] In this study, among the total fecal Enterobacteriales isolated, the majority were *E. coli*, followed by *K. pneumoniae*, *K. oxytoca*, and *Citrobacter* species. This finding is consistent with those of other investigations that found *E. coli* to be a common fecal isolate from transplant recipients,[17] outpatients,[18] healthy student populations,[3,19] school children,[5] and asymptomatic healthy individuals.[16,20]

The human fecal flora constitutes a potentially large reservoir of antibiotic-resistant bacteria at sites where resistance genes can be spread from intestinal commensals to potentially pathogenic bacterial species.[21] The *E. coli* isolates recovered from the stool samples displayed a greater percentage of resistance to the tested antibiotics and we have found 82.0% of the *E. coli* isolates resistant to ampicillin, 80.9% isolates resistant to amoxycillin-clavulanate, 66.3% to ofloxacin, 64.0% to ciprofloxacin, 60.7% to ceftazidime, 41.6% to meropenem, 39.3% to piperacillin-tazobactam, 38.2% to gentamicin, 37.1% to imipenem, 32.6% to tobramycin, and 30.3% to amikacin. Various studies from different countries have reported a varying antibiotic resistance rate of fecal *E. coli* isolates. Singh et al.[22] found a slightly higher rate of fecal *E. coli* isolates resistant to ampicillin, ceftazidime, and amikacin than our results and a lower rate of resistance to ciprofloxacin and ofloxacin in rural hill communities in Northeast India. In this study, 100% of the *Klebsiella* species were found resistant to amoxycillin-clavulanate, 58.3% to ceftazidime, 50.0% to ciprofloxacin and ofloxacin, 41.7% to imipenem, 33.3% isolates resistant to piperacillin-tazobactam, meropenem, gentamicin and amikacin, and 25.0% resistant to tobramycin. In contrast to the current study, Maharjan et al.[1] from Nepal found that *E. coli* and *Klebsiella* species isolated from the feces of healthy student volunteers had lower rates of resistance to ampicillin, piperacillin-tazobactam, ceftazidime, imipenem, ciprofloxacin, and gentamicin. This study revealed that the duration of transplantation also has a great impact on the antibiotic resistance pattern of fecal Enterobacteriales isolates. The isolates recovered from recipients who underwent a transplant procedure within six months at the time of sample collection exhibited an increased rates of resistance to all tested antibiotics than the isolates from recipients who underwent transplantation more than six months ago. This increased resistance in fecal Enterobacteriales may be possibly due to recent antibiotics consumption by the majority of renal transplant recipients, the use of intravenous antibiotics, and a history of admission to the hospital after transplantation where they may get colonized with antibiotic-resistant Enterobacteriales.

The chance of ESBL-E infection is higher in transplant recipients, which is most likely due to a combination of factors, particularly multiple antimicrobial prophylaxis for long periods of time and chronic immunosuppression.[1,6] In this study, 4.9% of fecal Enterobacteriales isolated from the stool samples of renal transplant recipients were found to be ESBL producers. The ESBL production was seen in 3.4% of *E. coli* isolates and 8.3% of *Klebsiella* species tested positive for ESBL. Our results on fecal carriage rate of ESBL-E among renal transplant recipients were nearly in range to the reports of Luvsansharav et al.[23] from Japan among healthy adult people (6.4%), Islam et al.[24] from USA among healthy children (3.5%), and Ebrahimi et al.[25] from Hungary among medical students (2.6%). Other studies from various countries reported varying percentages of fecal carriage of ESBL-E isolates among healthy populations. Kader et al.[16] from Saudi Arabia reported 13.1% fecal ESBL-E from asymptomatic healthy individuals and Sapkota et al.[3] from Nepal reported 29.5% ESBL-E fecal commensals among healthy student population. Previous studies have shown that among various groups of healthy populations, fecal carriage rates of ESBL-producing *E. coli* are reported to be 7.87% from Nepal,[1] 5.95% from United Kingdom,[26] 6.0 % from France,[27] 7.3% from Tunisia,[28] and 13.4% from Libya,[29] and ESBL-producing *Klebsiella* species are reported to be 20.5% and 9.7% from Nepal.[1,3] In a 10-year study in France (2001-2010), 4.1% of transplant recipients had fecal carriage of ESBL-E before transplantation, while

5.5% of recipients have developed ESBL-E infection within four months after transplantation, and in comparison to non-carriers, recipients who had pretransplant fecal carriage of ESBL-E were more likely to develop infection.[4] Similarly, in a meta-analysis of publications between 1994 and 2003, the pooled prevalence of 18% ESBL-E colonization was reported in SOT recipients.[30] In another study, colonization of CPE was observed in 2-18% of SOT recipients, whereas acquisition was found in 5-27% of recipients after transplantation.[31]

The ß-lactamase-producing fecal commensal Enterobacteriales isolated from healthy populations are increasingly becoming resistant to antibiotics.[3] When compared with the ß-lactamase non-producing Enterobacteriales, the ß-lactamase-producing isolates in this study showed a significant variation in their susceptibility to tested antibiotics and were found significantly more resistant to piperacillin-tazobactam (87.1% vs. 16.7%; *p-value<0.001*), imipenem (87.1% vs. 15.3%; *p-value<0.001*), meropenem (83.9% vs. 20.8%; *p-value<0.001*), gentamicin (71.0% vs. 22.2%; *p-value<0.001*), amikacin (64.5% vs. 15.3; *p-value<0.001*), and tobramycin (67.7% vs. 15.3%; *p-value<0.001*). It has also been noted in other studies from Nepal,[1,3,19] India,[32] and Japan[23] that ESBL-producing fecal Enterobacteriales exhibit a high degree of antibiotic resistance compared to that of ESBL-non-producing fecal Enterobacteriales. The widespread and extensive fecal carriage of antibiotic-resistant and ß-lactamase-producing Enterobacteriales in renal transplant recipients will almost undoubtedly pose a major threat to them.

This study did not investigate fecal colonization with Enterobacteriales before transplantation. The patient characteristics and risk factors associated with colonization with ESBL and MBL-producing Enterobacteriales were not studied. Due to financial constraints, the molecular analysis of the genes responsible for resistance in fecal Enterobacteriales was not undertaken.

CONCLUSIONS

This study reveals high rates of antibiotic resistance in fecal Enterobacteriales isolated from renal transplant recipients and the resistance rate is even higher in ß-lactamase-producing isolates. Each recipient should be screened for these highly resistant intestinal colonizers after renal transplantation in order to prevent possible infections in high-risk transplant recipients. In addition, transplant centers must have enhanced antimicrobial stewardship programs to reduce the incidence of infections caused by ESBL and MBL-producing Enterobacteriales. This may include measures such as promoting appropriate use of antibiotics, reducing unnecessary antibiotic use, and implementing strict infection prevention and control measures.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Maharjan A, Bhetwal A, Shakya S, Satyal D, Shah S, Joshi G, et al. Ugly bugs in healthy guts ! Carriage of multidrug-resistant and ESB-producing commensal Enterobacteriaceae in the intestine of healthy Nepalese adults. Infect Drug Resist. 2018;11:547–54.doi: https://doi.org/10.2147/IDR.S156593

2. Shi SH, Kong HS, Xu J, Zhang WJ, Jia CK, Wang WL, et al. Multidrug resistant gram-negative bacilli as predominant bacteremic pathogens in liver transplant recipients. Transpl Infect Dis. 2009;11(5):405–12.doi: https://doi.org/10.1111/j.1399-3062.2009.00421.x

3. Sapkota B, Yadav SK, Dhungana G, Ansari S, Mishra SK. Intestinal Carriage of Extended-Spectrum ß-Lactamase- (ESBL-) Possessing Escherichia coli and Klebsiella Species among Nepalese Health Science and Non-Health Science Students. Can J Infect Dis Med Microbiol. 2021;2021:1–7.doi: https://doi.org/10.1155/2021/4767429

4. Bert F, Larroque B, Paugam-Burtz C, Dondero F, Durand F, Marcon E, et al. Pretransplant Fecal Carriage of Extended-Spectrum ß-Lactamase-producing Enterobacteriaceae and Infection after Liver Transplant, France. Emerg Infect Dis. 2012;18(6):908–16.doi: https://doi.org/10.3201/eid1806.110139

5. Baral BP, Sharma BK, Gurung K, Gurung RR, Subramanava H. Fecal carriage of Extended Spectrum ß-Lactamases (ESBL) Producing Escherichia coli and Klebsiella spp. among School Children in Pokhara, Nepal producing Strains. Nepal J Biotechnol. 2018;6(1):26-32.doi: https://doi.org/10.3126/njb.v6i1.22334

6. Wang R, Han JH, Lautenbach E, Tamma PD, Thom KA, Alby K, et al. Clinical prediction tool for extended-spectrum beta-lactamase-producing enterobacterales as the etiology of a bloodstream infection in solid organ transplant recipients. Transpl Infect Dis. 2021;23(4):1-9.doi: https://doi.org/10.1111/tid.13599

7. Pérez-Nadales E, Fernández-Ruiz M, Gutiérrez-Gutiérrez B, Pascual Á, Rodríguez-Baño J, Martínez-Martínez L, et al. Extended-spectrum ß-lactamase-producing and carbapenem-resistant Enterobacterales bloodstream infection after solid organ transplantation: Recent trends in epidemiology and therapeutic approaches. Transpl Infect Dis. 2022;24(4):1–19.doi: https://doi.org/10.1111/tid.13881

8. Rodríguez-Baño J, Picón E, Gijón P, Hernández JR, Cisneros JM, Peña C, et al. Risk Factors and Prognosis of Nosocomial Bloodstream Infections Caused by Extended-Spectrum-ß-Lactamase-Producing Escherichia coli. J Clin Microbiol. 2010;48(5):1726-31.doi: https://doi.org/10.1128/JCM.02353-09

9. Isenberg H. Clinical Microbiology Procedure Handbook. Second Edi. American Society for Microbiology (ASM), Washington, DC, USA; 2007.

10. CLSI. Performance Standards for Antimicrobial Susceptibility Testing: M100 Document. 27th editi. Clinical and Laboratory Standards Institute, Wayne, PA, USA; 2017.

11. Mishra S, Acharya J, Kattel H, Koirala J, Rijal B, Pokhrel B. Metallo-beta-lactamase producing gram-negative bacterial isolates. J Nepal Health Res Counc [Internet]. 2012;10(22):208-13.[JNHRC]

12. So M, Walti L. Challenges of Antimicrobial Resistance and Stewardship in Solid Organ Transplant Patients. Curr Infect Dis Rep [Internet]. 2022;24(5):63-75. doi: https://doi.org/10.1007/s11908-022-00778-1

13. Camachoa AM, Camps IR. Nosocomial infection in patients receiving a solid organ transplant or haematopoietic stem cell transplant. Enferm Infecc Microbiol Clin. 2014;32(6):386-95.

14. Schwaber MJ, Klarfeld-lidji S, Navon-venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of Carbapenem-Resistant Klebsiella pneumoniae Acquisition among Hospitalized Adults and Effect of Acquisition on Mortality. Antimicrob Agents Chemother. 2008;52(3):1028-33.doi: https://doi.org/10.1128/AAC.01020-07

15. Cervera C, Delden C van, Gavaldà J, Welte T, Akova M, Carratalà J. Multidrug-resistant bacteria in solid organ transplant recipients. Clin Microbiol Infect. 2014;20(Supplement 7):49-73.doi: https://doi.org/10.1111/1469-0691.12687

16. Kader AA, Kumar A, Kamath KA. Fecal Carriage of Extended-Spectrum ß-Lactamase-Producing Escherichia coli and Klebsiella pneumoniae in Patients and Asymptomatic Healthy Individuals. Infect Control Hosp Epidemiol. 2007;28(9):1114-6. doi: https://doi.org/10.1086/519865

17. Bert F, Larroque B, Dondero F, Durand F, Paugam-Burtz C, Belghiti J, et al. Risk factors associated with preoperative fecal carriage of extended-spectrum ß-lactamase-producing Enterobacteriaceae in liver transplant recipients. Transpl Infect Dis. 2014;16(1):84-9.doi: https://doi.org/10.1111/tid.12169

18. Mandal DK, Sah SK, Mishra SK, Sharma S, Kattel HP, Pandit S, et al. Carriage of Extended-Spectrum-ß-Lactamase- and AmpC-ß-Lactamase-Producing Enterobacteriaceae (ESBL-PE) in Healthy Community and Outpatient Department (OPD) Patients in Nepal. Can J Infect Dis Med Microbiol. 2020;2020:1-9.doi: https://doi.org/10.1155/2020/5154217

19. Sharma B, Yadav SK, Khanal A, Shakya B, Shrestha K, Aryal S, et al. Fecal Carriage of Extended-Spectrum beta-Lactamase Producing Escherichia coli among Health Science Students. J Nepal Health Res Counc. 2022;20(54):160-5.

20. Babu R, Kumar A, Karim S, Warrier S, Nair SG, Singh SK, et al. Faecal carriage rate of extended-spectrum ß-lactamase-producing Enterobacteriaceae in hospitalised patients and healthy asymptomatic individuals coming for health check-up. J Glob

Antimicrob Resist. 2016;6:150-153.doi: https://doi.org/10.1016/j.jgar.2016.05.007

21. Nys S, Okeke IN, Kariuki S, Dinant GJ, Driessen C, Stobberingh EE. Antibiotic resistance of faecal Escherichia coli from healthy volunteers from eight developing countries. J Antimicrob Chemother. 2004;54(5):952-5.doi: https://doi.org/10.1093/jac/dkh448

22. Singh AK, Das S, Singh S, Gajamer VR, Pradhan N, Lepcha YD, et al. Prevalence of antibiotic resistance in commensal Escherichia coli among the children in rural hill communities of Northeast India. PLoS One. 2018;13(6):1-15.doi: https://doi.org/10.1371/journal.pone.0199179

23. Luvsansharav U-O, Hirai I, Niki M, Nakata A, Yoshinaga A, Yamamoto Y. Prevalence of fecal carriage of extended-spectrum ß-lactamase-producing Enterobacteriaceae among healthy adult people in Japan. J Infect Chemother. 2011;17(5):722-5.doi: https://doi.org/10.1007/s10156-011-0225-2

24. Islam S, Selvarangan R, Kanwar N, Mchenry R, Chappell JD, Halasa N, et al. Intestinal Carriage of Third-Generation Cephalosporin-Resistant and Extended-Spectrum ß-Lactamase-Producing Enterobacteriaceae in Healthy US Children. J Pediatric Infect Dis Soc. 2018;7(September):234-40. doi: https://doi.org/10.1093/jpids/pix045

25. Ebrahimi F, Mozes J, Monostori J, Goracz O, Fesus A, Majoros L, et al. Comparison of rates of fecal colonization with enterobacteria among patients in different wards, outpatients and medical students. Microbiol Immunol. 2016;60:285-94.doi: https://doi.org/10.1111/1348-0421.12373

26. Royden A, Ormandy E, Pinchbeck G, Pascoe B, Hitchings MD, Sheppard SK, et al. Prevalence of faecal carriage of producing Escherichia coli in veterinary hospital staff and students. Vet Rec Open. 2019;6(e000307):1-14.doi: https://doi.org/10.1136/vetreco-2018-000307

27. Nicolas-Chanoine M, Gruson C, Bialek-davenet S, Bertrand X, Thomas-Jean F, Bert F, et al. 10-Fold increase (2006-11) in the rate of healthy subjects with extended-spectrum ß-lactamase-producing Escherichia coli faecal carriage in a Parisian check-up centre. J Antimicrob Chemother. 2013;68(3):562-8.doi: https://doi.org/10.1093/jac/dks429

28. Sallem R, Slama K, Estepa V, Jouini A, Gharsa H, Klibi N, et al. Prevalence and characterisation of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli isolates in healthy volunteers in Tunisia. Eur J Clin Microbiol Infect Dis. 2012;31(7):1511-8.doi: https://doi.org/10.1007/s10096-011-1471-z

29. Ahmed SF, Ali MMM, Mohamed ZK, Moussa TA, Klena JD. Fecal carriage of extended-spectrum ß-lactamases and AmpC-producing Escherichia coli in a Libyan community. Ann Clin Microbiol Antimicrob. 2014;13(1):1-8.doi: https://doi.org/10.1186/1476-0711-13-22

30. Alevizakos M, Kallias A, Flokas M, Mylonakis E. Colonization with extended-spectrum beta-lactamase-producing Enterobacteriaceae in solid organ transplantation: A meta-analysis and review. Transpl Infect Dis. 2017;19(4):0-2.doi: https://doi.org/10.1111/tid.12718

31. Giannella M, Bartoletti M, Conti M, Righi E. Carbapenemase-producing Enterobacteriaceae in transplant patients. J Antimicrob Chemother. 2021;76(Supplement 1):i27-39.doi: https://doi.org/10.1093/jac/dkaa495

32. Mathai D, Kumar V, Paul B, Sugumar M, John K, Manoharan A, et al. Fecal Carriage Rates of Extended-Spectrum ß-Lactamase-Producing Escherichia Coli Among Antibiotic Naive Healthy Human Volunteers. Microb Drug Resist. 2015;19(1):59-64.doi: https://doi.org/10.1089/mdr.2014.0031