RESEARCH ARTICLE

Metronidazole Resistant *Bacteroides species* isolated from a Tertiary Care Hospital from North India

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ABSTRACT

Bacteroides species are the most common gram-negative anaerobic bacteria identified in surgical site infections as per our studies. Diabetic foot and burn wound infections have higher chances of surgeries and use of metronidazole for the treatment of anaerobic infections may lead to metronidazole resistance. There is a requirement for up-to-date geographical and therapeutically relevant data for *Bacteroides species*. The purpose of this study was to identify the rate of *B. species* with metronidazole resistance. We had collected 100 samples from burn wounds and diabetic foot ulcers in robertson cooked meat (RCM) broth. Then it was sub-cultured on anaerobic blood agar in a gas pak jar. Isolates were identified based on culture morphology and standard biochemical techniques. Antimicrobial sensitivity was evaluated by using several frequently used antimicrobial agents as per CLSI. A total of 52 anaerobic bacteria were isolated. In gram negative *B. species,* there were 34.61%, while in gram positive *Peptostreptococcus species,* there were 42.30%. 11.11% (4/18) of *Bacteroides species* were resistant by to metronidazole and had the same 11.11% (4/18) were resistant to piperacillin-tazobactum. In *Pepetostreptococcus* resistance to penicillin was about 72% (16/22) and 18% (2/22) in Metronidazole. *Eubacterium species* were 50% resistant to piperacillin-tazobactum and clindamycin. *Fusobactrium species* were totally resistant to penicillin.

Keywords: Anaerobic Infection, Burn wound Infections, Diabetic Foot.

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INTRODUCTION

Anaerobic bacterial infections are known to be associated with several human diseases, including intra-abdominal, vaginal tract, surgical sites, brain abscesses, and skin and soft tissue infections. According to studies, *B. species* were found in the majority of surgical site infections. Diabetes or burn patients, those who have recently had surgery, are more likely to have an anaerobic infection, especially by *B. species*.[1]

Metronidazole is a synthetic azomycin derivative, a nitroimidazole molecule derived from *Streptomyces* species, used to treat parasitic infections in the late 1950s. Metronidazole was developed in 1959, particularly to treat trichomoniasis. The antibacterial action of metronidazole was found in 1962. The medication has become a cornerstone in the treatment of anaerobic infections all over the world.[2]

Metronidazole resistance is caused by a number of molecular pathways, but in *B. species,* resistance is caused by nitroimidazole drug inactivation. *B. species* and *Helicobacter pylori* are the most widely researched anaerobes in terms of metronidazole resistance. This is unexpected given that resistance rates in the United Kingdom, Brazil, and Pakistan are dangerously high (5–10%). Despite having the largest breadth of known resistance to antimicrobial drugs among anaerobes, the global rate of metronidazole resistance remains modest, at 5%.[3]

Because of their indiscriminate use, failure to recognize and control the infectious process may have devastating consequences like limb amputation, sepsis, and mortality. The evolution of metronidazole resistance in *B. species* can reduce the drug's effectiveness.[1]

Metronidazole-resistant *B. species* are mainly found in patients treated with long term therapy with metronidazole.[1] Isolation and susceptibility testing of anaerobic bacteria are

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typically not conducted in developing countries because of inadequate laboratory capability. As a result, therapy is mainly empirical, and if adequate anti-anaerobic drugs are not utilized, then the treatment will become rigid. Resistance in *B. species* against metronidazole is increasing day by day. There is a lack of data against resistance in *B. species* towards metronidazole. As a result, there is a requirement to assess the metronidazole resistant *B. species* isolated from patients.

The present study was designed to determine the frequency of *B. species* as well as its resistance to metronidazole from burn wound and diabetic foot ulcer infections.

MATERIAL AND METHOD

A one-year prospective study was conducted at the Department of Microbiology at Maharishi Markandeshwar Institute of Medical Science and Research, Mullana, Haryana state of India. A total of 100 samples were collected from burn wounds and diabetic foot ulcers in RCM broth. The ethical approval was taken from Institutional Ethical Committee. Criteria for inclusion Samples were obtained from a patient with diabetic foot ulcers and burn wounds. All samples other than burn wounds and diabetic feet were excluded. The pus sample was obtained in accordance with normal practice in RCM broth.

Microscopy

The Gram stain of the samples was inspected under a microscope at 100x and the results were documented.

Anaerobic Bacteria Identification

After 24 hours of incubation on blood agar enriched with haemin and vitamin K, L-cysteine, and yeast extract with preparatory discs such as metronidazole (5 g), vancomycin (5 g), and colistin (10 g) Sodium Polyanethol Sulphonate (SPS) discs, RCM was subcultured. Incubation for anaerobiasis was performed in a gas pack jar as per the standard method. RCM was saved for backup cultures. Further, conventional processing was used to generate pure isolates.[4]

Identification of Anaerobic Bacteria

Isolated pure cultures were identified by culture morphology and standard biochemical techniques.[4] The disc diffusion method was used to evaluate antimicrobial resistance using several frequently used antimicrobial agents that are approved by Clinical Laboratory Standard Institute 2017.[5] for anaerobes bacterial antibiotic susceptibility patterns.

Anaerobic Bacteria were Subjected to the Following Antibiotics

Penicillin, Clindamycin, Piperacillin-tazobactam, Amoxyclavulanic acid, Imipenem, Metronidazole

RESULTS

In this study, 100 wound swabs were collected from June 2017 to May 2018, from patients with burn wounds and diabetic foot infections with their consent. A 52 anaerobic bacteria were isolated, 63.46% of which were from male patients and 36.53% were from female patients. The highest culture positivity was seen in patients from rural areas, i.e., 76.92 and 23.07% positivity in patients from urban areas. The age distribution of isolates also revealed that the majority of the anaerobic bacteria isolated were from the 21–50 age group, i.e., 80.76%, followed by patients younger than 20 (11.53%) and older than 50 (7.6%). A 28 (53.84%) anaerobic bacteria were isolated from patients with diabetic foot infections and 24 (46.15%) burn wound infections (Table 1). Polymicrobial anaerobic growth with aerobic bacteria was observed in 76% cases.

In the presented study, common gram-positive anaerobic isolates were *Peptostreptococcus species (*42.30%), followed by *Eubacterium species (*19.23%), while in gram negative isolates, *B. species* (34.61%), followed by *Fusiform bacterium species* 3.84%. (Table 2).

Data on Antimicrobial Susceptibility

B. species were resistant by 11.11% (4/18) to metronidazole and had the same 11.11% (4/18) resistance to piperacillin-tazobactum (Table-3). Resistance to penicillin was about 72% (16/22) in *Pepetostreptococcus species*, while it was 18% in Metronidazole. *Eubacterium species* have shown 50% resistance to piperacillin-tazobactum and clindamycin. *Fusobactrium species* have complete resistance to penicillin 2/2.

DISCUSSION

B. species are one of the major anaerobic bacteria isolated from surgical site infections studied in Japan. Bacterial resistance is increasing day by day as well as in anaerobic bacteria due to overuse of antibiotics and use for prolonged periods to treat infections empirically. There is a lack of studies on metronidazole resistant *B. species* in developing countries.

So we designed this study to determine the frequency of *B. species* and other anaerobic bacteria with their resistance profile to metronidazole from burn wound and diabetic foot ulcer infections. The positive rate was 52% in this study. Males were shown to have higher anaerobic culture positivity,

Table 1: Demographic Profile with positivity rate

S. No	*Variables*	*N= 52*	*Positivity (%)*
1	Male	33	64.46
2	Females	19	36.53
3	Urban	12	23.07
4	Rural	40	76.92
5	>20 age	6	11.53
6	21–50 age	42	80.76
7	<50 age	4	7.6
8	Diabetic foot infections	28	53.84
9	Burn wound infections	24	46.15

Table 2: Anaerobic bacteria isolated from clinical Samples

Gram reactions	*Name of isolates*	*N = 52*	*%*
Gram positive	*Peptostreptococcus species*	22	42.30
	Eubacterium species	10	19.23
Gram negative	*Bacteroides species*	18	34.61
	Fusiform bacterium species	02	3.84

Table 3: Resistance Profiles of Anaerobic Bacteria Isolated

Organism (N = 52)	*Peptostreptococcus species (N = 22)*	*Bacteroides species (N = 18)*	*Eubacterium Species (N = 10)*	*Fusiform bacterium Species (N = 02)*
Metronidazole	02	4	NT	0
Vancomycin	0	NT	0	NT
Colistin	NT	0	NT	0
Penicillin	16	NT	5	2
Clindamycin	NT	0	5	NT
Piperacillin-tazobactum	NT	4	0	NT
Imipenem	0	0	NT	0

NT- Not Tested

i.e., 63.46%, than females, i.e., 36.53%. Garg R *et al*. (2021) [6] observed a lower positivity rate than our study. For anaerobic isolates, after one week of dressing, the chances might be increased. Other studies[6-10] have reported similar findings. Approximately 76% of culture media shows polymicrobial growth shown similar results, which is common in both infections. Polymicrobial growth with aerobic bacteria was very high, even though in some cultures we isolated two aerobic bacteria with one anaerobic bacteria (data not included). Age plays a major role in diabetic foot infections. We have 80% of patients from the 21 to 50 age group, and culture positivity was high in 76.92% of rural-origin patients because we have most of the patients from rural areas. *Peptostreptococcus* species (42.30%), followed by Eubacterium species (19.23%), were the most common gram-positive anaerobic isolates, while *B. species* (34.61%), followed by Fusiform bacterium 3.84%, were the most common gram-negative isolates. The findings were in concordance with studies done by Garg *et al*.,[9] Eslami *et al*.,[10] and Murphy *et al*.[11] While there are studies which show *Bacteroides fragilisis is* the predominant anaerobic bacteria (Table 2).[12,13] *B. species* were resistant by 11.11% (4/18) to metronidazole. Other studies also observed similar results, like India and Croatia having 24.5%, followed by Canada, South Africa, and Ontario with 8, 2.9, and 1.2%, respectively, resistant to metronidazole[14-17] and had the same 11.11% (4/18) resistance to piperacillin-tazobactum (Table 3). Resistance to penicillin was about 72% (16/22) in *Pepetostreptococcus species*, while it was 18% in Metronidazole. *Eubacterium species* have shown 50% resistance to piperacillin-tazobactum and clindamycin. *Fusobactrium species have* complete resistance to penicillin 2/2. Resistance to Metronidazole and penicillin showed less resistance in our study as compared to a study done by Garg R *et al*.,[9] Cobo *et al*.,[20] Pednekar *et al*.,[18] Reymundo *et al*.,[19] and Cobo *et al*.[20] Geographical regions and seasons play a major role in the bacterial resistance profile. Prolonged use of broad-spectrum antibiotics leads to resistance in anaerobic bacteria.

Here are the few limitations of this study we want to acknowledge: the study was done with a limited number of samples and isolated only a few organisms without their specificity level, and we used the disc diffusion method to assess resistance to metronidazole and other antibiotics instead of the dilution method, which gives minimum inhibitory concentration (MIC). Antibiotic resistance is increasing day by day and is also seen in anaerobes. We can also identify another antibiotic or any compound which shows a synergic effect with metronidazole to overcome the resistance. On the other hand, there are a few genes and enzymes responsible for resistance to metronidazole in the Indian population. I hope we will see antibiotic stewardship for anaerobic bacterial infections in all the hospitals in India, which is helpful to decrease the resistance to antibiotics.

CONCLUSION

Metronidazole resistance *B. species* was detected in our study**.** But Metronidazole is the most active in vitro agent against *B. species.* It was concluded that regular monitoring of the resistance profile against anaerobes in different geographical regions was critical to providing an appropriate treatment profile and more precisely preventing infection. There are genes that also play a major role in the transmission of metronidazole resistance in a healthy person, and Antibiotic prescriptions on the basis of antibiotic resistance profiles can avoid indiscriminate use of antibiotics, which helps prevent infection and transmission of resistance in anaerobic bacteria.

REFERENCES

1. Noor A, Khetarpal S. Anaerobic Infections. [Updated 2021 Aug 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan.
2. Cunningham, F. E., D. M. Kraus, L. Brubaker, and J. H. Fischer. 1994. Pharmacokinetics of intravaginal metronidazole gel. J. Clin. Pharmacol. 34:1060-1065.
3. Alauzet C, Lozniewski A, Marchandin H. Metronidazole resistance and nim genes in anaerobes: A review. Anaerobe. 2019 Feb;55:40-53.
4. Jousimies-Somer HR, Summanen P, Citron DM, Baron EJ, Wexler HM, Finegold SM. Wadsworth-KTL anaerobic bacteriology manual. 6th ed. Belmont, California: Star Publishing Company, 2003.
5. CLSI. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard – 27 Edition, M100 standard 2017
6. Garg R, Kumari N, Chauhan S. Identification and antimicrobial susceptibility testing of anaerobes isolated from clinical specimens: a study re-emphasizing role of the neglected bacteria causing significant infections. JK Science 2021;23(2):60-63.
7. mal W, Al Hashem G, Rotimi VO. Antimicrobial resistance among anaerobes isolated from clinical specimens in Kuwait

hospitals: comparative analysis of 11-year data. Anaerobe 2015;31:25-30.10.
8. De A, Gogate A. Prevalence of gram negative anaerobic bacilli in routine clinical specimens. Indian J Pathol Microbiol2001;44(4):435-438
9. Garg R, Datta P, Gupta V, Chander J. Anaerobic bacteriological profile of infected diabetic foot ulcers with their antimicrobial susceptibility pattern: need of the hour. Natl J Lab Med 2017;6(3):MO01-04.12.
10. Eslami G, Fallah F, Goudarzi H, Navidinia M. The prevalence of antibiotic resistance in anaerobic bacteria isolated from patients with skin infections. Gene Ther Mol Biol 2005;9:263-68.13.
11. Murphy EC, Frick IM. Gram-positive anaerobic cocci -commensals and opportunistic pathogens. FEMS Micro biol Rev 2013;37:520-553
12. Saini S, Gupta N, Aparna, Lokveer, Griwan MS. Surgical infections: a microbiological study. Braz J Infect Dis2004;8(2):118-25.15.
13. Akhi MT, Ghotaslou R, Beheshtirouy S, Asgharzadeh M,Pirzadeh T, Asghari B, *et al*. Antibiotic susceptibility pattern of aerobic and anaerobic bacteria isolated from surgical site infection of hospitalized patients. Jundishapur J Microbiol 2015;8(7): e20309
14. Marchand-Austin A, *et al*. Antimicrobial susceptibility of clinical isolates of anaerobic bacteria in Ontario, 2010–2011. Anaerobe. 2014;28:120-125.
15. Novak A, *et al*. Antimicrobial susceptibility of clinically isolated anaerobic bacteria in a university hospital Centre Split, Croatia in 2013. Anaerobe. 2015;31:31-36.
16. Meggersee R, Abratt V. The occurrence of antibiotic resistance genes in drug resistant Bacteroides fragilis isolates from Groote Schuur hospital, South Africa. Anaerobe. 2015;32:1-6.
17. Chaudhary M, Payasi A. Emerging metronidazole resistance in anaerobes and mapping their susceptibility behavior. Am J Infect Dis. 2014;10(2):56.
18. Pednekar SN, Pol SS, Kamble SS, Deshpande SK,Bharadwaj RS. Drug resistant anaerobic infections: are they complicating diabetic foot ulcer? Int J Healthc Biomed Res 2015;3(3):142-48.21.
19. Raymundo MF, Mendoza MT. The microbiologic features and clinical outcome of diabetic foot infections among patients admitted at UP-PGH. Philipp J Microbiol Infect Dis 2002;31(2):54 -63.22.
20. Cobo F, Borrego J, Gómez E, Casanovas I, Calatrava E,Foronda C, *et al*. Clinical findings and antimicrobial susceptibility of anaerobic bacteria isolated in blood stream infections. Antibiotics (Basel) 2020;9(6):345.