ANTIMICROBIAL RESISTANCE OF ANAEROBIC BACTERIA ISOLATED AT VIET DUC UNIVERSITY HOSPITAL

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Summary

Objectives: To evaluate the antibiotic susceptibility of anaerobic bacteria strains isolated from clinical specimens at Viet Duc University Hospital. Subjects and ***methods:*** A descriptive study and lab analysis on 167 anaerobic isolates isolated from clinical specimens. ***Results:*** The highest rate of resistance to the isolates was observed for metronidazole (84.43%), followed by clindamycin (49.7%) and antibiotics in the carbapenem group (2.99% - 8.98%), respectively. The lowest rate of resistance was shown for piperacillin/tazobactam (1.2%). However, the rate of resistance to antibiotics was variable in isolated strains. In more detail, amoxicillin/clavulanate varied from 0.00% - 10.53%, piperacillin/tazobactam (0.00% - 2.17%), metronidazole (78.95 % - 93.75%), carbapenems (0.00% - 12.50%), moxifloxacin (0.00% - 10.53%), and clindamycin (40.00% - 85.42%). The susceptibility to metronidazole was 80.43% in 46 *Bacteroides* isolates and 4.17% in 48 *Prevotella* isolates. Compared to other antibiotics, the resistance to metronidazole is significantly high, specifically for *Prevotella,* with the highest rate of 93.75%. ***Conclusion:*** The rate of resistance to antibiotics in pathogenic anaerobic bacterial isolates fluctuated with amoxicillin/clavulanate (0.00% - 10.53%), piperacillin/tazobactam (0.00% - 2.17%), carbapenems (0.00% - 12.50%), moxifloxacin (0.00% - 10.53%), and clindamycin (40.00% - 85.42%). The resistance varied in different strains. *Prevotella* and *Micromonas* were shown to be less resistant to antibiotics than *Bacteroides* and *Clostridium*.

** Keywords: Antimicrobial resistance; Anaerobic bacteria.*

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Date received: 08/02/2023

Date accepted: 22/3/2023

http://doi.org/10.56535/jmpm.v48i4.289

INTRODUCTION

For aerobic bacteria, monitoring and managing the rational use of antibiotics is of concern at all levels. However, the status of anaerobic bacteria infections and their antibiotic resistance have not been given due attention. *Bacteroides fragilis* is a common cause of resistance to almost antibiotics, including carbapenem and metronidazole [1, 2]. Regularly monitoring the antibiotic resistance of anaerobic pathogens becomes necessary [3, 4]. Therefore, this study aimed to: *Evaluate the level of resistance of anaerobic bacteria strains isolated at Viet Duc University Hospital.*

SUBJECTS AND METHODS

1. Subjects

167 isolates of anaerobic bacteria were isolated and identified at Viet Duc University Hospital.

* *Materials and equipment:*

- Culture media: Columbia blood agar supplemented with vitamin K and haemin.

- System Whitley anaerobic Workstations; model: DG250.

- MICRONAUT-Wilkins-Chalgren Broth (E2-330-020) of BD.

- The tube contains 1 - 2 mL of 0.9% NaCl (pH 5.5 - 6.5) (E2-312-001) of BD.

- Standard 0.5. McFarland tube

2. Methods

* *Study design:* A descriptive study and lab analysis.

Experiments were performed on a MICRONAUT tray. 200 µL of 0.5 McFarland anaerobic bacterial suspension was added into a MICRONAUT broth tube. On the MICRONAUT tray 96 wells, each well containing lyophilized antibiotics in different concentrations, 100 µL of bacterial broth was added into the well, and the mixture was incubated in an anaerobic atmosphere for 24 - 48 hours at 37°C. Different concentrations of antibiotics were prepared in different wells. The results are determined by observing the turbidity or transparency (clarity) of the wells on the MICRONAUT tray.

The susceptibility test with MICRONAUT plates is based on the rehydration of antibiotics after adding a standardized bacterial suspension. The results were done with a plate reader and data were evaluated and counted from MICRONAUT reads.

* *Analyze and process data:*

Research data were processed using SPSS Statistic 25.0 software. Descriptive statistics are presented as frequencies (n) and percentages (%).

RESULTS AND DISCUSSION

Table 1: Antibiotic resistance rate of anaerobic bacteria (n = 167 isolates).

Antibiotics	n	Number and rate (n, %)			MIC		
		Susceptible (S)	Intermediate (I)	Resistant (R)	S	I	R
Amoxicillin/ Clavulanate	167	154 (92.22)	5 (2.99)	8 (4.79)	≤ 4/2	8/4	≥ 16/8
Piperacillin/ Tazobactam	167	130 (77.84)	35 (20.96)	2 (1.20)	≤ 16/4	42/4 - 64/4	≥ 128/4
Metronidazole	167	23 (13.77)	3 (1.80)	141 (84.43)	≤ 8	16	≥ 32
Imipenem	167	152 (91.02)	5 (2.99)	10 (5.99)	≤ 4	8	≥ 16
Meropenem	167	146 (87.43)	6 (3.59)	15 (8.98)	≤ 4	8	≥ 16
Ertapenem	167	159. (95.21)	3 (1.80)	5 (2.99)	≤ 4	8	≥ 16
Moxifloxacin	167	146 (87.43)	5 (2.99)	16 (9.58)	≤ 2	4	≥ 8
Clindamycin	167	78 (46.71)	6 (3.59)	83 (49.70)	≤ 2	4	≥ 8

n: the total number of bacterial isolates;

MIC: minimum inhibitory concentrations.

The highest resistance rate among 167 isolates was observed for metronidazole (84.43%), followed by clindamycin (49.70%), respectively. The resistance rate to carbapenem antibiotics ranged from 2.99 - 8.98%. The lowest antibiotic resistance rate in bacterial isolates was 1.20% for piperacillin/tazobactam. In general, the rate of antibiotic resistance tends to increase, especially for metronidazole.

Table 2: Antibiotic resistance rate of Bacteroides (n = 46 isolates).

Antibiotics	n	Number and rate (n,%)		
		Susceptible (S)	Intermediate (I)	Resistant (R)
Amoxicillin/Clavulanate	46	45 (97.83)	0 (0.0)	1 (2.17)
Piperacillin/Tazobactam	46	44 (95.65)	1 (2.17)	1 (2.17)
Metronidazole	46	9 (19.57)	0 (0.0)	37 (80.43)
Imipenem	46	45 (97.83)	0 (0.0)	1 (2.17)
Meropenem	46	43 (93.48)	1 (2.17)	3 (4.35)
Ertapenem	46	45 (97.83)	0 (0.0)	1 (2.17)
Moxifloxacin	46	41 (89.13)	1 (2.17)	4 (8.70)
Clindamycin	46	33 (71.74)	1 (2.17)	12 (26.09)

The rate of antibiotic resistance ranged from 2.17 - 4.35% in the *Bacteroides* group of the following antibiotics: Carbapenem and amoxicillin/clavulanate, piperacillin/tazobactam groups, especially for metronidazole, the high resistance rate of this group was 80.43%, for clindamycin was 26.09%.

The rate of antibiotic resistance of anaerobic bacteria strains in our study was higher than that in a study by Tran Lan Phuong (2000) [5], in which *Bacteroides fragilis* was 100% susceptible to metronidazole, 85.7% to imipenem, and amoxicillin/clavulanate, respectively. Audrey (2014) showed that the group *Bacteroides fragilis* was resistant to piperacillin/tazobactam with a rate of less than 10%, and the rate of resistance to the carbapenem antibiotic group was less than 1.5%. The rate of resistance to amoxicillin-clavulanate, carbapenem, and metronidazole of the *Prevotella* group was less than 10%. According to the author, the rate of metronidazole resistance was rare [6]. A study in Canada (2012) reported in 387 isolates of *Bacteroides fragilis* collected from 9 hospitals that the rate of metronidazole resistance was 0.3%; piperacillin-tazobactam was 0.5%; imipenem was 0.8%; ertapenem was 2.3%; doripenem was 8.0%; amoxicillin-clavulanate was 2.7%, respectively [7].

Table 3: Antibiotic resistance rate of Prevotella (n = 48 isolates).

Antibiotics	n	Number and rate (n,%)		
		Susceptible (S)	Intermediate (I)	Resistant (R)
Amoxicillin/Clavulanate	48	42 (87.50)	4 (8.33)	2 (4.17)
Piperacillin/Tazobactam	48	19 (39.58)	29 (60.42)	0 (0.00)
Metronidazole	48	1 (2.08)	2 (4.17)	45 (93.75)
Imipenem	48	41 (85.42)	3 (6.25)	4 (8.33)
Meropenem	48	40 (83.33)	2 (4.17)	6 (12.50)
Ertapenem	48	45 (93.75)	2 (4.17)	1 (2.08)
Moxifloxacin	48	45 (93.75)	1 (2.08)	2 (4.17)
Clindamycin	48	7 (14.58)	0 (0.00)	41 (85.42)

The rate of antibiotic resistance of 48 *Prevotella* isolates ranged from 0 - 12.50% for the following antibiotics: Carbapenem and amoxicillin/ clavulanate, piperacillin/tazobactam groups, especially the resistance rate to metronidazole was highest (93.75%), followed by clindamycin (85.42%).

Table 4: Antibiotic resistance rate of *Clostridium* (n = 19 isolates).

Antibiotics	n	Number and rate (n,%)		
		Susceptible (S)	Intermediate (I)	Resistant (R)
Amoxicillin/Clavulanate	19	17 (89.47)	0 (0.00)	2 (10.53)
Piperacillin/Tazobactam	19	18 (94.74)	1 (5.26)	0 (0.00)
Metronidazole	19	3 (15.79)	1 (5.26)	15 (78.95)
Imipenem	19	18 (94.74)	1 (5.26)	0 (0.00)
Meropenem	19	18 (94.74)	0 (0.00)	1 (5.26)
Ertapenem	19	19 (100.00)	0 (0.00)	0 (0.00)
Moxifloxacin	19	16 (84.21)	1 (5.26)	2 (10.53)
Clindamycin	19	10 (52.63)	1 (5.26)	8 (42.11)

The antibiotic resistance rate ranged from 0 - 10.53% of 19 *Clostridium* isolates to the following antibiotics: Carbapenems and amoxicillin/clavulanate, piperacillin/ tazobactam groups, especially for metronidazole, the high resistance rate was 78. 95%, with clindamycin was 42.11%, respectively.

Compared to the results from Tran Thi Lan Phuong, *Clostridium perfringens* was 80% susceptible to metronidazole, 90 - 100% to imipenem, amoxicillin/ clavulanate [5], respectively; however, the antibiotic resistance rate of 19 *Clostridium* isolates in our research was higher. Other studies also showed a lower rate of antibiotic resistance than our results, such as: According to Audrey (2014), the *Clostridium* group was susceptible to piperacillin, carbapenem antibiotics, metronidazole, and vancomycin. However, *Clost idium difficille* is usually susceptible to metronidazole and vancomycin but resistant to beta-lactam antibiotics, fluoroquinolones, and clindamycin [6].

Table 5: Antibiotic resistance rate of *Micromonas micros* (n = 10 isolates).

Antibiotics	n	Number and rate (n,%)		
		Susceptible (S)	Intermediate (I)	Resistant (R)
Amoxicillin/Clavulanate	10	10 (100.00)	0 (0.00)	0 (0.00)
Piperacillin/Tazobactam	10	10 (100.00)	0 (0.00)	0 (0.00)
Metronidazole	10	2 (20.00)	0 (0.00)	8 (80.00)
Imipenem	10	9 (90.00)	0 (0.00)	1 (10.00)
Meropenem	10	10 (100.00)	0 (0.00)	0 (0.00)
Vancomycin	10	8 (80.00)	0 (0.00)	2 (20.00)
Moxifloxacin	10	9 (90.00)	1 (10.00)	0 (0.00)
Clindamycin	10	5 (50.00)	1 (10.00)	4 (40.00)

Out of 10 *Micromonas micros* isolates, the rate of antibiotic resistance to amoxicillin/clavulanate, piperacillin/tazobactam, and meropenems was not observed (0.0%), but the rate for metronidazole was 80% and for clindamycin was 40%, respectively.

CONCLUSION

The data showed that anaerobic bacteria strains isolated in this study were resistant to different antibiotics at various degrees. The resistance rates of 167 anaerobic pathogenic bacteria isolate fluctuated with amoxicillin/ clavulanate (0.00% - 10.53%), piperacillin/ tazobactam (0.00% - 2.17%), carbapenems (0.00% - 12.50%), moxifloxacin (0.00% - 10.53%), and clindamycin (40.00% - 85.42%). The highest rate was 93.75% for metronidazole.

REFERENCES

1. Karen C. Carroll, et al. (2019). Manual of clinical microbiology.

2. Sutter, V., D. Citron, S. Finegold (1980). Wadsworth Anaerobic Bacteriology Manual 3rd end. St Louis: CV Mosby.

3. Garcia, L., H. Isenberg. (2007). Aerobic bacteriology. Clinical microbiology procedures handbook. update. *ASM Press*. Washington C, D.

4. Tang, Y.-W., et al. (2015). Molecular Medical Microbiology–The Expanding Concept, in Molecular Medical Microbiology. *Elsevier;* 1-4.

5. Phương, T.T.L. (2003). Nghiên cứu về vi khuẩn trong dịch mật của bệnh nhân sỏi đường mật và mức độ nhạy cảm với kháng sinh của chúng. *Luận văn bác sĩ chuyên khoa cấp II.* Đại học Y Hà Nội.

6. Audrey N. Schuetz1. (2014). Antimicrobial Resistance and Susceptibility Testing of Anaerobic Bacteria.

7. Karlowsky, J.A., et al. (2012). Prevalence of antimicrobial resistance among clinical isolates of Bacteroides fragilis group in Canada in 2010 - 2011: CANWARD surveillance study. *Antimicrob Agents Chemother*; 56(3): 1247-1252.