

Original Research Article

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Emerging Non Fermentative Gram Negative Bacteria and their Antibiogram from Various Clinical Samples Collected from Patient Admitted to Medical Intensive Care Units

Anirudh*, Anil Bilolikar and Sukrutha Gopal Reddy

Department of Microbiology, Krishna Institute of Medical Sciences, Minister Road,
Secunderabad - 500003, T.S., India

*Corresponding author

ABSTRACT

Keywords

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Non fermentors are important nosocomial pathogens causing opportunistic infections in immunocompromised patients. They cause infections with patients having humidifiers, ventilators and catheter devices especially in medical intensive care units. To isolate identify in which age group, sex, risk factors of non fermentative gram negative bacteria The study was performed from September 2018 to February 2019 for a period of 6 months in Department of Microbiology & Medical Intensive Care Units. The samples were inoculated on Blood agar, Mac Conkey agar and incubated at 37°C for overnight. Non-lactose fermentation was noted on MacConkey agar. The isolates were identified and Antimicrobial susceptibility testing was done by using GN card and AST N281 respectively by using Vitek 2 compact (Biomerieux). A total of 3143 clinical samples were obtained from medical intensive care units. 100 NFGNB were isolated with prevalence of 3.18. Males are more predominant than females. The most common age group in which non fermenters were isolated is 61-70 years followed by 51-60 years. Among the *Acinetobacter baumannii* (n=40) maximum sensitivity is seen in colistin followed by tigecycline. And among *Pseudomonas aeruginosa* (n=38) maximum sensitivity is for colistin and gentamycin.

Introduction

Non fermenting gram negative bacteria (NFGNB) are non sporing, aerobic bacteria which do not ferment glucose and and have positive cytochrome oxidase test and usually they do not grow on MacConkey agar¹. They account for 10-15% of all bacterial isolates and cause life threatening infections such as

septicemia, surgical site infection, wound infection, meningitis, osteomyelitis and pneumonia².

Majority of non fermentating gram negative bacteria emerged as important nosocomial pathogens causing opportunistic infections in immune compromised patients³. The source of establishing infections for these non

fermentating gram negative bacteria are dialysis units, humidifiers, ventilator machines, catheter devices⁴. The predominant non fermentors are *Pseudomonas aeruginosa*, *Acinetobacter baumannii* followed by *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Elizabethkingae*, *Chryseobacterium* and *Alcaligenes* species. Routinely they are identified only in a few laboratories in India as they grow slowly and require special culture media and other biochemical tests for identification⁵. *Pseudomonas aeruginosa* infection is prevalent among patients with, acute leukemia, organ transplants and intravenous injections. Infections usually tends to accumulate where moisture occurs in sites such as tracheostomy site, indwelling catheters, burns, the external ear and weeping cutaneous wounds. Intensive care units(ICU) are more prone to vulnerable population and the emergence of non fermenting gram negative bacteria and their resistance to several antibiotics has raised a severe issue in predicting patient treatment outcomes.

The aim of the study is to identify in which age group, sex and risk factors are present to, isolate, identify and characterize, prevalence of non fermenting Gram negative bacteria and their antibiotic sensitivity from various clinical samples in Medical Intensive Care Units. Among the NFGNB isolated most common was *Acinetobacter baumannii* (40) followed by *Pseudomonas aeruginosa* (38), *Elizabethkingia meningoseptica* (7), *Burkholderia cepacia* (4), *Stenotrophomonas maltophilia* (4), *Ralstonia picketti* (2), *Pseudomonas* spp (2), *Sphingomonas* spp (3).

Materials and Methods

It was a prospective, analytical, observational study performed in the Krishna Institute of Medical Sciences. The study was performed from September 2018 to February 2019 for a

period of 6 months in Department of Microbiology & Medical Intensive Care Units. The samples were inoculated on Blood agar, Mac Conkey agar and incubated at 37° for overnight. Non-lactose fermentation was noted on MacConkey agar. Preliminary identification of non fermenting gram negative bacteria was done by Gram staining, motility, oxidase and catalase test.

The isolates were identified and Antimicrobial susceptibility testing was done by using GN card and AST N281 respectively by using Vitek 2 compact (Biomerieux). The quality control of GN card (identification testing) was done by using ATCC 700323 *Enterobacter hormaechei*, ATCC 17666 *Stenotrophomonas maltophilia*, ATCC 700327 *Enterococcus casseliflavus*, ATCC BAA 750 *Staphylococcus saprophyticus*. The quality control of AST N281 card (Antimicrobial susceptibility testing) was done by using ATCC 25922 *Enterococcus faecalis*, ATCC 27853 *Pseudomonas aeruginosa*, ATCC 35218 *Escherichia coli*. Antimicrobial susceptibility testing in which MIC values were reported as per CLSI 2018 & 2019 guidelines²⁹.

Results and Discussion

A total of 3143 clinical samples were obtained from medical intensive care units. 100 NFGNB were isolated with prevalence of 3.18. Males n=77(77%) and females were n =23(23%). Males are more predominant than females.

The most common age group in which non fermenters were isolated is 61-70 years followed by 51-60 years,71-80 years and 81-90 years.

Among the NFGNB isolated most common was *Acinetobacter baumannii* (40) followed by *Pseudomonas aeruginosa* (38),

Elizabethkingia meningoseptica (7), *Burkholderia cepacia* (4), *Stenotrophomonas maltophilia* (4), *Ralstonia picketti* (2), *Pseudomonas* spp (2), *Sphingomonas* spp (3).

Among the *Acinetobacter baumannii* (n=40) maximum sensitivity is seen in colistin followed by tigecycline and maximum resistance is piperacillin tazobactam, amikacin and aztreonam. And among *Pseudomonas aeruginosa* (n=38) maximum sensitivity is for colistin and gentamycin.

Among the risk factors, most common risk factor for isolation of NFGNB is catheter associated and more number of isolates are presented with more than 5 risk factors.

Non fermenters are intrinsically resistant to various antimicrobials and are known to produce Extended spectrum beta lactamases (ESBL's) and metallobeta lactamases (MBL's).

In the present study maximum NFGNB were isolated in the age group of 61-70years(29%) which is comparable to study done by Mushtaq⁶ *et al.*, where maximum number of NFGNB are obtained in the age group above 60 years 21(39.6%). This could be due to comorbidities present in the geriatric age group, making them prone to diseases due to lesser immunity. This differs from studies conducted by Maniyan⁷ *et al.*, showing maximum number of non fermenters isolated in age group of less than 10 years 23(20.91%) followed by 21-30 years 21(19.09%) and least in age 60 years 21(19.09%).

In this study, NFGNB are observed more in males (77%) than in females (23%), which correlates to studies done by Ridhima⁸ *et al.*, where NFGNB isolated in males and females were 69.7% and 30.3% respectively. In a

study conducted by Kalidas⁹ *et al.*, NFGNB were isolated in 55% males and 45% females. In the present study, the prevalence of NFGNB was 3.18%, which is comparable to Benachinmardi¹⁰ *et al.*, (3.5%) and Bruno¹¹ *et al.*, (2.18%). In studies conducted by Bhuvaneshwari G¹², Khante¹³ *et al.*, the prevalence was 6.85% and 8.67% respectively. This is due to demographic and epidemiological variation of other studies mentioned as they have included all the intensive care units because of which their rate of NFGNB isolation is high. The low isolation rate in our study may be due to strict infection control practices and implementation of antibiotic stewardship programme.

In the present study maximum number of isolates are obtained from Endotracheal secretion (50%) followed by Sputum (19%), bronchial wash (8%) and blood(8%). The study is comparable to study done by Mushtaq⁶ *et al.*, and Harris *et al.*, where respiratory samples accounted for 39.6% and 50% respectively.

The organisms isolated are *Acinetobacter baumannii* (40%), followed by *Pseudomonas aeruginosa* (38%), *Elizabethkingae meningoseptica* (7%), *Burkholderia cepacia* (4%), *Stenotrophomonas maltophilia* (4%), *Ralstonia picketti* (2%), *Sphingomonas paucimobilis* (2%), *Pseudomonas putida*(1%), *Pseudomonas luteola* (1%), *Sphingomonas spiritivorum*(1%).

In the present study *Acinetobacter baumannii* (40%) is the most common isolate followed by *Pseudomonas aeruginosa*(38%). This is comparable from study done by Samanta¹⁴ *et al.*, where *Acinetobacter baumannii* and *Pseudomonas aeruginosa* accounted for 66% and 26% of the isolates respectively.

Table.1 The various antibiotics and their minimal inhibitory concentration (MIC) interpretive criteria with susceptible, intermediate and resistant MICs for *NFGNB* as given by CLSI (2018-2019) as mentioned in the table.

S.No.	Name of the Antibiotic	MIC Interpretive Criteria (µg/MI)		
		S	I	R
01	Ticarcillin+Clavulanic acid	≤ 16	32-64	≥128
02	Piperacillin+Tazobactam	≤16	32-64	>128
03	Ceftazidime	≤8	16	≥32
04	Cefaperazone+Sulbactam	≤8	-	≥32
05	Cefepime	≤8	16	≥32
06	Aztreonam	≤8	16	≥32
07	Doripenem	≤2	4	≥8
08	Imipenem	≤2	4	≥8
09	Meropenem	≤2	4	≥8
10	Amikacin	≤16	32	≥64
11	Gentamycin	≤4	8	≥16
12	Ciprofloxacin	≤0.5	1	≥2
13	Levofloxacin	≤1	2	≥4
14	Minocycline	≤4	8	≥16
15	Tigecycline	≤2	4	≥8
16	Colistin	≤2	-	≥4
17	Cotrimoxazole	≤40	-	≥80

Fig.1 Age wise distribution of patients

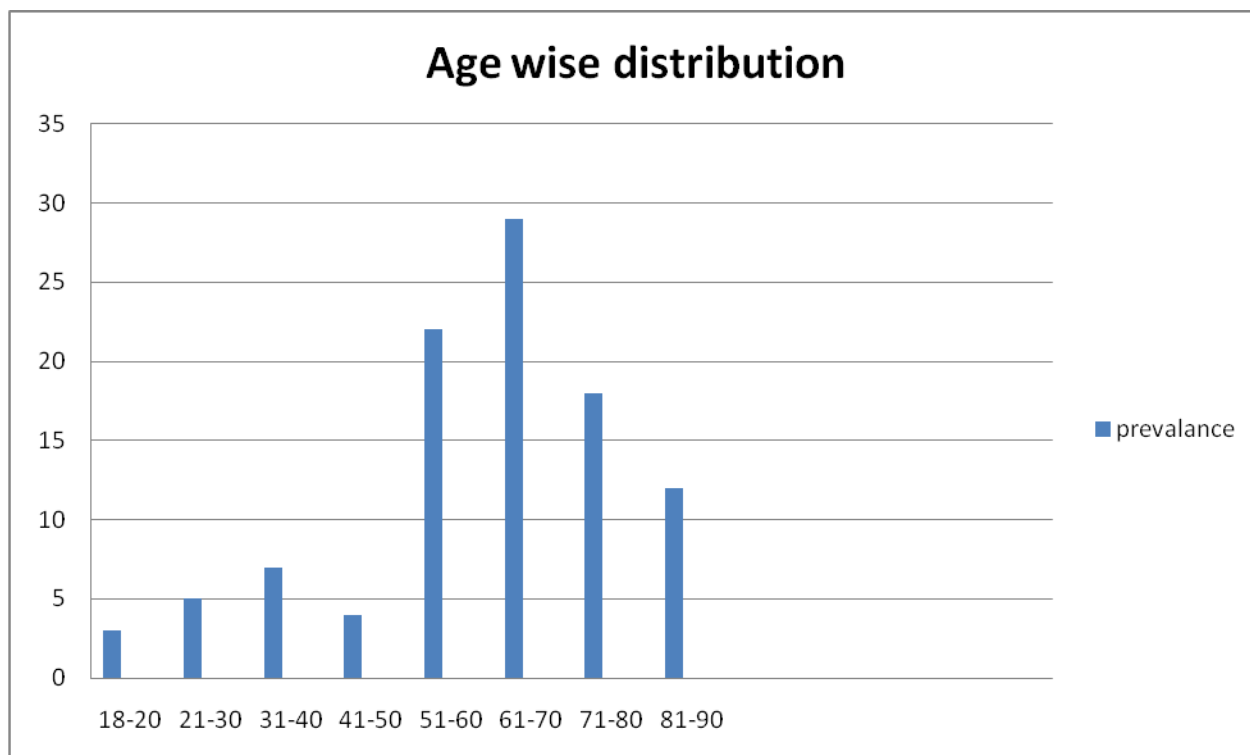


Table.2 Age wise distribution of patients in whom NFGNB were isolated.

Age group	18-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90
prevalance	3(3%)	5(5%)	7(7%)	4(4%)	22(22%)	29(29%)	18(18%)	12(12%)

Table.3 Prevalance of NFGNB from various clinical samples .

Organism	Et secretion	Blood	Urine clean	Urine catheter catch	Pus	Bronchial wash	Sputum	Body fluids	Catheter tip	Total
<i>Pseudomonas aeruginosa</i>	15	2	2	1	3	1	12	1	1	38
<i>Acinetobacter baumannii</i>	21	2	0	0	2	6	6	2	1	40
<i>Burkholderia cepacia</i>	2	2								4
<i>Elizabethkingae meningoseptica</i>	6	1								7
<i>Ralstonia picketti</i>	1	1								2
<i>Stenotrophomonas maltophila</i>	3					1				4
<i>Sphingomonas paucimobilis</i>							1		1	2
<i>Pseudomonas luteola</i>	1									1
<i>Pseudomonas putida</i>				1						1
<i>Sphingomonas spiritivorum</i>	1									1
Total	50	8	2	2	5	8	19	3	3	100

Table.4 Antibiotic susceptibility pattern of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* to various antibiotics tested

Antibiotic	<i>Pseudomonas aeruginosa</i> (n=38)			<i>Acinetobacter baumannii</i> (n=40)		
	sensitive	intermediate	resistant	sensitive	intermediate	resistant
Ticarcillin+ clavunilic acid	14(36.8%)	7(18.4%)	17(44.7%)	2(5%)		38(95%)
Piperacillin Tazobactam	21(55.2%)	2(5.2%)	15(39.4%)	1(2.5%)		39(97.5%)
Ceftazidime	26(68.4%)	2(5.2%)	10(26.3%)	2(5%)		38(95%)
Cefaperazone sulbactam	24(63.1%)	3(7.8%)	11(28.9%)	3(7.5%)	5(12.5%)	32(80%)
Cefepime	26(68.4%)	2(5.2%)	10(26.3%)	2(5%)		38(95%)
Aztreonam	17(44.7%)	5(13.1%)	16(42.1%)	1(2.5%)		39(97.5%)
Doripenem	25(65.7%)	3(7.8%)	10(26.3%)	2(5%)		38(95%)
Imipenem	26(68.4%)		12(31.5%)	2(5%)		38(95%)
Meropenem	24(63.1%)	1(2.6%)	13(34.2%)	1(2.5%)	1(2.5%)	38(95%)
Amikacin	27(71.1%)	1(2.6%)	10(26.3%)	1(2.5%)		39(97.5%)
Gentamycin	28(73.6%)	1(2.6%)	9(23.6%)	3(7.5%)	2(5%)	35(87.5%)
Ciprofloxacin	23(60.5%)	1(2.6%)	14(36.8%)	2(5%)		38(95%)
levofloxacin	21(55.2%)		17(44.7%)	2(5%)	1(2.5%)	37(92.5%)
Minocycline				18(45%)	4(10%)	18(45%)
Tigecycline				37(92.5%)	2(5%)	1(2.5%)
Colistin	38(100%)			40(100%)		
Cotrimoxazole						

Table.5 Antibiotic susceptibility pattern of *Burkholderia cepacia* and *Stenotrophomonas maltophilia* to various antibiotics tested.

Antibiotic	<i>Burkholderia cepacia</i> (n=4)			<i>Stenotrophomonas maltophilia</i> (n=4)		
	S	I	R	S	I	R
Ticarcillin+ Clavunalic acid			4(100%)	4(100%)		
Ceftazidime	4(100%)			3(75%)		1(25%)
Meropenem	2(50%)	1(25%)	1(25%)	NA	NA	NA
levofloxacin	1(25%)	2(50%)	1(25%)	4(100%)		
Minocycline	2(50%)	1(25%)	1(25%)	4(100%)		
Cotrimoxazole	3(75%)		1(25%)	4(100%)		

Table.6 Antibiotic susceptibility pattern of *Elizabethkingae meningoseptica* to various antibiotics tested

Antibiotic	<i>Elizabethkingae meningoseptica</i> (n=7)		
	S	I	R
Rifampicin	6(85.7%)		1(14.2%)
Cotrimoxazole	6(85.7%)		1(14.2%)
Ciprofloxacin	4(57.1%)	1(14.2%)	2(28.5%)
Clindamycin	5(71.4%)		2(28.5%)
vancomycin	7(100%)		
Minocycline	7(100%)		

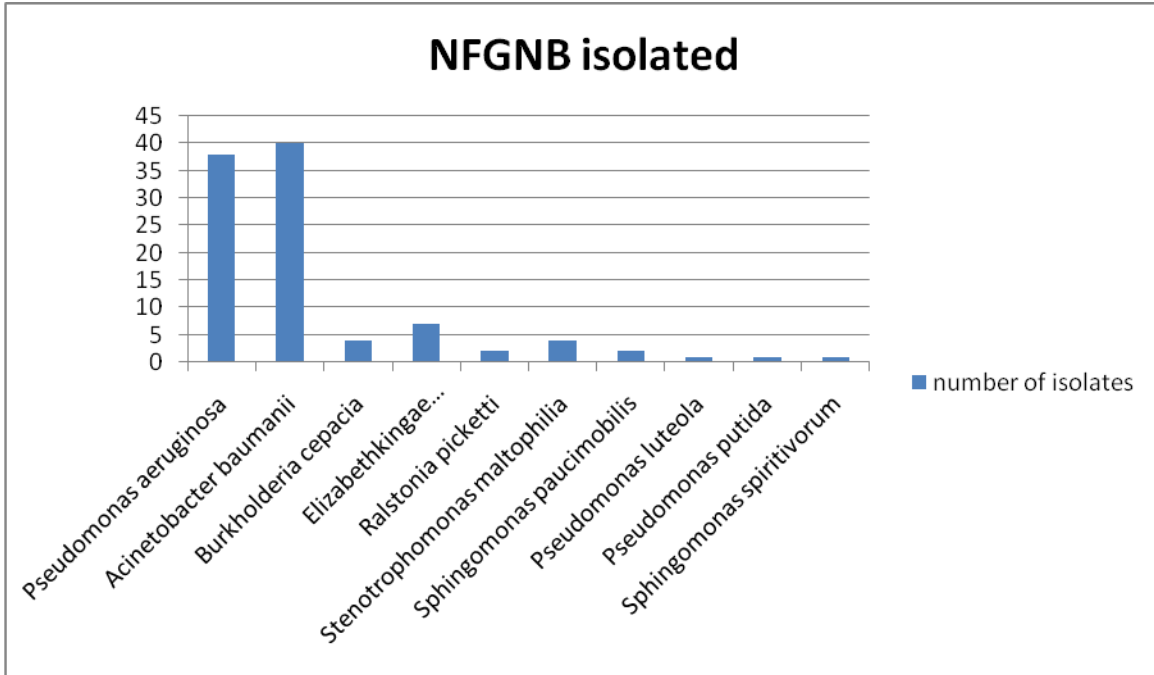
Table.7 Number of Risk factors in study population.

Total no of patients	1 risk factor	2 risk factors	3 risk factors	4 risk factors	5 risk factors	6 risk factors	7 risk factors
100	1	9	21	17	24	22	6

Table.8 Risk factor wise distribution of patients of NFGNB isolated

Risk factor	Number of patients
Hypertension	47
Diabetes	52
Catheter associated	98
Iv canula associated	75
Central line associated	64
Ventilator associated	75
wound associated	34

Fig.2 Total number of NFGNB isolated



However, this differs from studies done by Eltahawy and Khalaf¹⁵ where *Acinetobacter baumannii* and *Pseudomonas aeruginosa* accounted for 34% and 31% respectively. Also, in studies conducted by Wang H¹⁶ *et al.*, *Acinetobacter baumannii* was isolated in 56% of the isolates, followed by *Pseudomonas aeruginosa* (46.9%). These studies are more of western in origin and they have considered all the intensive care units whereas our study is only medical intensive care units.

Among Endotracheal secretions, the *Acinetobacter baumannii* was the most common isolate (42%), followed by *Pseudomonas aeruginosa* (30%). These findings are similar to study done by Benachinmardi¹⁰ *et al.*, where the most common isolate among ET secretions was *Acinetobacter baumannii* (41%). Also, in a study conducted by Maniyan⁷ *et al.*, *Acinetobacter baumannii* accounted for 80% of ET secretion isolates and *Pseudomonas aeruginosa* accounted for 20%. On the contrary, this study differs from a study

conducted by Sudha Krishnan¹⁷ *et al.*, where the isolation rate of *Acinetobacter baumannii* is 36.7% and *Pseudomonas aeruginosa* is 53.9%.

Among the sputum samples, most number of isolates are *Pseudomonas aeruginosa* (63.1%) followed by *Acinetobacter baumannii* (31.5%). These findings correlate with Benachinmardi¹⁰ *et al.*, Khante¹³ *et al.*, Kaur¹⁸ *et al.*, where *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were 43% and 78% respectively. In a study conducted by Malini² *et al.*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* accounted for 23% and 16.3% of the sputum isolates, respectively.

In the study, the antibiotic sensitivity of *Acinetobacter baumannii* to colistin is 100% and to tigecycline is 92.5%. Similarly, in a study by Nazir¹⁹ *et al.*, sensitivity of *Acinetobacter baumannii* to colistin is 100% to tigecycline (84%). However, the findings of Baruah²⁰ *et al.*, showed variation from present study to colistin susceptibility (12.5%).

In the study *Acinetobacter baumannii* showed maximum resistance to Piperacillin Tazobactam (97.5%), Amikacin (97.5%) and Aztreonam (97.5%). Study done by Memish²¹ *et al.*, showed maximum resistance to Aztreonam (95.5%) and Amikacin (76.9%) and study done by Kaur¹⁸ *et al.*, showed maximum resistance to Amikacin (90%) and Piperacillin tazobactam (87%). Whereas, in a Study done by Jithendranath²² *et al.*, Maniyan⁷ *et al.*, there was least resistance to Piperacillin tazobactam (15% and 27% respectively). A study done by Prasanna²³ *et al.*, there was least resistance to piperacillin tazobactam (4.3%) and Amikacin (2.6%).

In the study, *Pseudomonas aeruginosa* showed 100% sensitive to Colistin (100%) and Gentamycin (73.6%) which is similar to studies done by Parajuli²⁴ *et al.*, Khante¹³ *et al.*, Prasanna²³ *et al.*, Kaur¹⁸ *et al.*, where *Pseudomonas aeruginosa* is sensitive to colistin (100%), (100%), (95.3%), (100%) and gentamycin were (62.5%), (52%), (94.7%), (55%) respectively.

Pseudomonas aeruginosa is intrinsically resistant to Minocycline, Tigecycline and Cotrimoxazole as per CLSI 2018 guidelines.

In the study, *Pseudomonas aeruginosa* is maximum resistant to levofloxacin (44.7%). It is similar to study done by Khante¹³ *et al.*, where levofloxacin (44.87%). In the study, *Pseudomonas aeruginosa* is maximum resistant to ticarcillin+clavunilic acid (44.7%) and it is similar to study conducted by Juyal²⁵ *et al.*, where ticarcillin+ clavunilic acid is (30%). In the present study *Pseudomonas aeruginosa* are resistant to several antibiotics because of low permeability of the outer membrane, constitutive expression of various efflux pumps, and production of antibiotic inactivating enzymes.

Elizabethkingae meningoseptica has also been

reported to cause infections in patients receiving hemodialysis and healthcare-associated bacteriuria primarily in elderly patients with diabetes.

Elizabethkingae meningoseptica is maximum sensitive to vancomycin (100%) and Minocycline (100%). It is comparable to study done by Tak²⁶ *et al.*, Sinha²⁷ *et al.*, showing isolate is sensitive to Vancomycin. Fraser²⁸ *et al.*, showed that the isolate is maximum sensitive to minocycline but resistant to vancomycin which is contraindicating from the present study.

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