



## **Antibiotics Susceptibility Pattern of Hospital Indoor Airborne Bacteria in Hawassa University Teaching and Referral Hospital, South Ethiopia**

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**Abstract:** Nosocomial infection (NI) is an infection acquired whilst staying, visiting or working in a hospital or healthcare facility. It is also defined as an infection acquired in hospital by a patient who was admitted for a reason other than that infection or an infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission. One of the risk factors for such infection is bacterial contamination of hospital wards indoor air by bacteria. In view of that, the microbiological quality of air can be considered as a mirror of the hygienic condition for hospital wards. A cross sectional study was conducted from May to August 2011 in central triage, emergency, surgical outpatient, medical outpatient, gynecology and obstetrics, pediatrics, surgical inpatient and medical outpatient wards of Hawassa Teaching Referral Hospital to assess antibiotic susceptibility of the indoor airborne bacteria in selected hospital wards. Air samples of the selected wards were collected using Settle Plate Method in the morning and in the afternoons and cultured aerobically. Of the 128 indoor air samples collected from the hospital rooms, in which 153 isolates were isolated. High level of antimicrobial resistance was observed among the isolates obtained in this study; 86.9% were resistant to 1 or more antimicrobials and 73.8 were multidrug resistant. Resistance was especially high against amoxicillin (64.9%) and penicillin (58.4%). Hospital management was advised to reduce foot trafficking and to periodically assess the quality of indoor air to identify and minimize/eliminate sources of microbial contamination.

**Key words:** Bio-aerosols, Indoor environments, Nosocomial infections, Settle Plate method

### **1. Introduction**

The term airborne indicates that a pathogen is spread through the air. Airborne pathogens have the potential to spread more rapidly. Air that is contaminated with a microorganism is called a microbial aerosol. With microbial aerosol, air becomes the vehicle to transmit pathogens. Bio-aerosols are airborne particles that are living (bacteria, viruses and fungi) or originate from living organisms. Bio-aerosols are ubiquitous, highly variable, complex, natural or man-made in origin <sup>[1]</sup>. Airborne pathogens are those pathogens that are generated in the respiratory system and transferred in the air as a way of propagation <sup>[2]</sup>. Atmospheric pollution is one of the most pressing problems of our age. This pollution has now reached advanced level that poses a potential threat to the health and wellbeing of the population <sup>[3]</sup>. The atmosphere consists of different components, which enhance or promote the survival of microorganisms in the air. It is composed of 75% nitrogen, 21% oxygen, 0.9% argon, 0.03% carbon dioxide and 0.076% other trace gases, very low concentration of organic and inorganic nutrients and free water at an irregular intervals <sup>[4]</sup>. Exposure to bio-aerosols, containing airborne microorganisms and their byproducts, can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions <sup>[5,6]</sup>.

The health and wellbeing of the public are affected by the physical, chemical and biological properties of the indoor environment. The quality of the indoor environment, however, is not easily defined or controlled and can potentially place human occupants at risk <sup>[4]</sup>. Hospital indoor air contains a diverse range of

microbial population. The significance of these microbes is debatable in some quarters, where as elsewhere it may be considered significant. In many environments including hospitals, animal sheds, clean-rooms, pharmaceutical facilities and spacecraft environments, the presence of bio-aerosols can compromise normal activities, making efficient monitoring crucial <sup>[7-9]</sup>. Microbial damage in indoor/outdoor area is caused most frequently by molds and bacteria. These microorganisms have a very important role in the biogeochemical cycle, as their task consists of disintegrating organic mass to reusable metabolites. In the environment spores of molds and bacteria may become airborne and are therefore ubiquitous. They can enter indoor areas either by means of passive ventilation or by means of ventilation systems <sup>[10]</sup>.

Environmental degradation and hazards such as indoor air pollution, droughts, floods, and poor sanitation are closely associated with Ethiopia's relatively high prevalence of respiratory diseases (acute respiratory infections, lung cancer, and asthma). A number of empirical studies confirm the links between exposure to indoor air pollution and the incidence of respiratory diseases and a variety of peri-natal health hazards arguably due to maternal exposure during pregnancy <sup>[11]</sup>. Indoor environments are favorable spaces for airborne microorganisms. The presence of moisture from various sources make the possibility for broad distribution. Every living creature (microscopic, animal, human) contributes to airborne contamination in the surrounding environments ([WWW.technology-forum.com/fileadmin/](http://WWW.technology-forum.com/fileadmin/)). Bio-aerosols contribute to about 5 to 34% of indoor air pollution. The source of bio-

aerosols in indoor air includes furnishing and building materials, microbiological contamination within the walls and ceilings and floor activities. Other significant sources of airborne indoor bacteria are occupants [12]. The immediate environment of man comprises of air on which depends all forms of life. The bacterial content of air breathed is important particularly when the air contains pathogenic organisms since a man respire about 15m<sup>3</sup> of air in a day. Higher levels of contamination are observed when there are many occupants, much bodily movements or dust raising activities [13]. Air contains significant number of microorganisms, acting as a medium for their transmission or dispersal. Inhalation, ingestion and dermal contact are the routes of human exposure to airborne microorganisms, inhalation being the predominant. Airborne microorganisms are diverse and can be harmless, but some are highly pathogenic. Many lethal airborne bacteria, fungi and viruses affect human beings. Legionellosis, pneumonia, tuberculosis, meningitis, scarlet fever, anthrax, SARS or flu are only a few examples of current airborne diseases [14].

Although, every effort is made to kill or check the growth of microorganisms in the hospital, the hospital environment is a major reservoir for variety of pathogens. One reason is that certain normal microbiotas of the human body are opportunistic and present a particularly strong danger to hospital patients. In fact the microbes that cause nosocomial infection may not cause disease in healthy people, but are pathogenic to individuals whose defenses have been weakened by illness or therapy. In addition to being opportunistic, some microorganisms in hospital become resistant to antimicrobial drugs, which are commonly used there [15].

The importance of the estimation of the quantity and types of airborne microorganisms is that, they can be used as an index for the cleanliness of the environment as well as an index they bear in relation to human health and as source of hospital acquired infections [3]. The source and spread of organisms inside the hospital are important issues. Human related organisms or the body normal flora, also found in clothing, are spread through shedding during human activities. In indoor environments, the main source for microbes is usually the outdoor air. In addition to outdoor sources, microbes indoors can originate from indoor sources. These can be the occupants themselves and their activities. Exposure to bio-aerosols may be especially hazardous in clinics and hospitals where they may be a major factor in increasing morbidity from respiratory diseases [3]. Human responses to bio-aerosols range from innocuous effects to serious diseases depending on the exposure and the susceptibility of human beings to it (e.g., genetic factors, age, personal habits, duration of hospital stay, medication) [16].

One of the more alarming recent trends in infectious diseases has been the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial infection [17]. Resistance to

antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections (NIs) as well as for resistant community acquired infections. As resistance develops to first-line antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs. The development of antibiotic resistant bacterial strains is a serious threat to present hospital care practice [18].

There are several reports on the prevalence of NI in Africa; Algeria 4% [19], Tunisia 13% [20], and Morocco 6.7% [21] and 17.8% [22]. A study conducted in 1988 on 1006 surgical patients admitted to Tikur Anbessa hospital, in Addis Ababa, revealed a prevalence of NIs of 16.4%. Wound (59%), urinary tract (26%), and respiratory tract (6%) infections accounted for more than 90% of these infections [23]. According to [24], the susceptibility patterns of isolates causative to NI revealed varying degrees of resistance to the antibiotics tested. *Staphylococcus aureus* showed 100% resistance to methicillin, 78% to ampicillin, 71.5% to penicillin and the least resistance which is 9.6% was observed for ciprofloxacin. On the other hand, *S. aureus* isolates were 100% sensitive for vancomycin. Ceftriaxone was relatively effective antibiotic for the treatment of pathogens which were responsible to cause NIs.

## 1.2 Significance of the study

Good environmental condition at working place is necessary for health of workers and for the quality of the work. Hygiene aspects play a notable role in the everyday functioning of a hospital especially in premises where medical processes critical to vital functions are carried out or where the patient is very vulnerable to infections. Indoor air quality is more critical in health care facilities than in most other indoor environments because of the many dangerous microbial and chemical agents present. The complex hospital environment requires special attention to ensure healthful IAQ to protect patients and healthcare workers against HAI (NIs). Hospital indoor air contains a diverse range microbial population. These microbes include bacteria, fungi and others pathogens, which are causative agents of Nosocomial infection.

## 1.3 Objectives of the study

### 1.3.1 General objective:

- To assess antibiotics susceptibility patterns of hospital indoor air borne bacteria isolated from Hawassa Referral teaching Hospital.

### 1.3.2 Specific objectives:

- ❖ To identify the type of indoor airborne bacteria at the Hawassa Teaching and Referral Hospital.
- ❖ To test the antimicrobial susceptibility of the isolates against commonly used antimicrobials.

- ❖ To identify the drug of choice among commonly used drugs

## 2. Materials and Methods

### 2.1 Description of the study area

The study was conducted from May to August 2011 at Hawassa Teaching and Referral Hospital. Hawassa is the capital city of the Southern Nations Nationalities and Peoples Regional State (SNNPRS), located on the shores of Lake Hawassa in the Great Rift Valley, 275 km South of Addis Ababa. The city is located at coordinate points of 7°2'N and 38°28'E, at an elevation of 1708 meters above sea level (Local History of Ethiopia, 2007). Hawassa Teaching and Referral hospital is a government hospital which serves the population of Hawassa and Southern parts of the country encompassing roughly 17 million people. This Hospital has started service in 2005 and is attached to Hawassa University. The hospital has 350 beds for admitted patients. Currently, there are about 720 staffs involved in teaching (on different disciplines of health profession), hospital service and administration.

### 2.1 Sampling technique and sample size

Purposive sampling was used in selecting wards and then random sampling technique using lottery method was used to select rooms from selected wards of the hospital. Sample size was determined on convenience, a total of 128 settle plate samples were collected from 16 rooms belonging to 8 wards under two departments of the hospital as summarized in Table 2. Samples were collected for consecutive 2 weeks for 2 days per week and twice a day (in the morning between 10Am and 11Am and in the afternoon between 1PM and 2PM), making it 8 settle plate samples from each single selected room of the selected wards. Therefore, a total of 128 samples were collected from 16 rooms representing 8 selected wards under two departments.

### 2.3 Sample collection and transportation

Air sampling was performed with settle plate method. Settle plate samples of indoor air from the selected rooms of the hospital were collected without controlling any indoor environmental condition. The air samples were collected by exposing blood agar plates, in the air labeled with room number, time and date of sample collection and then transported to selected rooms and placed lid open at 1 metre above the ground for 40 minutes and then the plates were covered with their lids and taken to Microbiology laboratory of Collage of Medicine and Health Science located in the premises of the hospital and incubated aerobically for 24 - 48 hrs at 37°C to allow the growth of bacteria. The air sample was taken two times: in the morning between 10Am and 11Am and in the afternoon between 1PM and 2 PM twice per week for consecutive two weeks. This time of sampling (morning and afternoon) was selected since human activity increase at this time.

### 2.4 Identification of the isolates

Isolates obtained after incubation were initially characterized by colony morphology, hemolysis on blood agar, microscopic appearance after Gram staining,

growth and characteristic on selective and indicator media. The isolates were then sub cultured on appropriate culture media and further identification was done using biochemical tests such as catalase, urease, motility (SIM), coagulase, indole production, citrate utilization and characteristics on triple sugar iron agar (TSI) to identify the isolates to genera/species levels.

### 2.5 Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates for commonly used antimicrobials was determined with modified Kirby-Bauer agar disk diffusion method on Mueller-Hinton agar (MHA) media. All isolates obtained during the study were tested against six commonly used antibiotics namely amoxicillin (AML, 2µg), norfloxacin (NOR, 10µg), vancomycin (VA, 30µg), gentamycin (CN, 10µg), penicillin G (P, 10µg) and ceftriaxone (CRO, 30µg) (Oxoid, Basingstoke, England). 0.5 McFarland standard suspension was used for standardization of bacterial suspensions.

The zone of complete growth inhibition around each of the disc was carefully measured using transparent plastic ruler, and the zone diameter was compared to a standard table for that drug and concentration. The isolates were then reported as susceptible, intermediate or resistant according to interpretative chart of complete growth inhibition zone diameter sizes for bacteria using the modified Kirby-Bauer disk diffusion technique (WHO, 2003; NCCLS, 2006).

### 2.6 Data analysis

Data were entered and managed in Microsoft Excel and data analysis was performed by Stata version 9 (Stata Corp. College Station, TX) statistical software. Descriptive statistics was used to summarize and present the quantity and types of airborne bacteria. Comparisons were made using Chi-square test. A P-value of < 0.05 was considered statistically significant.

## 3. Results

### 3.1 Bacterial Isolates

Nine species/genera of bacteria were isolated from the 128 settle plate samples examined in the study. *Staphylococcus aureus* and *Klebsiella* spp. were the most frequently isolated bacteria, 30.5 % and 29.7% of the exposed plates being positive for them respectively while *Enterobacter*, *Citrobacter* and *E. coli* were the least frequent with 3.1, 4.7 and 5.5% prevalence respectively. *Staphylococcus aureus* and *Klebsiella* spp. were consistently isolated from the eight different hospital wards investigated.

### 3.2 Antimicrobial susceptibility patterns of the isolates

The antimicrobial susceptibility of the 153 isolates recovered in this study against commonly used 6 antibiotics (AML, P, CRO, CN, VA and NOR) is summarized in Table 8. Of the total 153 isolates (when they were considered irrespective of their species) 133 (86.9%) were resistant to 1 or more antibiotics and 113 (73.8%) were resistant to 2 or more antimicrobials. The highest resistance was recorded against amoxicillin

(64.7%) followed by penicillin (62.1%), ceftriaxone (41.2%), norfloxacin (35.9%), vancomycin (20.3%) and gentamycin (15.7%), while one of Klebsiella isolate was resistant to all six tested drugs.

The gram positive isolates were more resistant to amoxicillin where 63.6% were resistant to the drug, followed by penicillin (57.6%), ceftriaxone (42.4%) and norfloxacin (36.4%). However, they were sensitive to vancomycin (84.6%) and gentamicin (80.3%). Similar to this, the gram negative isolates were more resistant to for both amoxicillin and penicillin (65.5%), followed by

ceftriaxone (40.2%) and norfloxacin (35.6%). They were sensitive to gentamicin (79.3%) and vancomycin (69.0%). Gram positive isolates (63.6%) were more resistant to norfloxacin compared to gram negative isolates (35.6%) ( $P < 0.01$ ), while gram negative isolates were more resistant to vancomycin (31.0% versus 6.1%) ( $P < 0.001$ ) and gentamicin (19.5% vs. 10.6%) ( $P < 0.05$ ) compared to gram positive isolates. High antimicrobial resistance of *S. aureus* was recorded for penicillin (61.5%), amoxicillin (53.9%) and ceftriaxone (51.3%). However, few isolate of

**Table 1:** Antimicrobial susceptibility of bacterial isolates recovered from indoor air samples of rooms at the hospital

Isolates (N=153)	Status*	Antimicrobial agents**					
		AML n (%)	CRO n (%)	P n (%)	NOR n (%)	VA n (%)	CN n (%)
<i>S. aureus</i> (n=39)	I	3 (7.7)	15 (38.5)	3 (7.7)	0 (0.0)	3 (7.7)	6 (15.4)
	R	21 (53.9)	20 (51.3)	24 (61.5)	12 (30.8)	0 (0.0)	3 (7.7)
	S	15 (38.5)	4 (10.3)	12 (30.8)	27(69.2)	36(92.3)	30 (79.9)
<i>Klebsiellaspp</i> (n=38)	I	0 (0.0)	16 (42.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	21 (55.3)	15 (39.5)	23 (60.5)	18 (47.4)	7 (18.4)	5 (13.2)
	S	17 (44.7)	7 (18.4)	15 (39.5)	20(55.6)	31 (81.6)	33 (86.8)
<i>Proteus spp</i> (n=18)	I	0 (0.0)	4 (22.2)	6 (33.3)	9 (50.0)	0 (0.0)	0 (0.0)
	R	13 (72.2)	9 (50.0)	12 (66.7)	6 (33.3)	9 (50.0)	5 (27.8)
	S	5 (27.8)	5 (27.8)	0 (0.0)	3 (16.7)	9 (50.0)	13 (72.2)
CNS (n=16)	I	4 (25.0)	12 (75.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	10 (62.5)	0 (0.0)	3 (18.8)	9 (56.3)	0 (0.0)	0 (0.0)
	S	2(12.5)	4(25)	13(81.3)	7(43.7)	16(100)	16 (100)
<i>Serratiaspp</i> (n=14)	I	0 (0)	2(14.3)	0(0)	3(21.4)	0 (0)	0 (0)
	R	10 (71.4)	5 (35.7)	10 (71.4)	7 (50.0)	0 (0)	4 (28.6)
	S	4(28.57)	7(50)	4(28.57)	4(28.6)	14(100)	10 (71.4)
Streptococci Spp (n=11)	I	0(0)	3(27.3)	0(0)	0(0)	3(27.3)	0 (0)
	R	11(100)	8(72.7)	11(100)	3(27.3)	4(36.4)	4 (36.4)
	S	0(0)	0(0)	0(0)	8(72.7)	4(36.4)	7 (63.7)
<i>E. coli</i> (n=7)	I	0(0)	5(71.4)	1(14.3)	2(28.6)	0(0)	0(0)
	R	5(71.4)	2(28.6)	4(57.1)	0(0)	4(57.1)	1(14.3)
	S	2(28.6)	0(0)	2(28.6)	5(7.4)	3(42.9)	6(85.7)
<i>Citrobacter</i> (n=6)	I	2(33.3)	4(66.7)	0(0)	2(33.3)	0(0)	1(16.7)
	R	4 (66.7)	2 (33.3)	4 (66.7)	0 (0)	4 (66.7)	2 (33.3)
	S	0(0)	0(0)	2(33.3)	4(66.7)	2(33.3)	3(50)
<i>Enterobacter spp</i> (n=4)	I	0(0)	1(25)	0(0)	0(0)	0(0)	0(0)
	R	4(100)	2(50)	4(100)	0(0)	3(75.0)	0(0)
	S	0(0)	1(25)	0(0)	4(100)	1(25)	4(100)
Total (N=153)	I	9(5.9)	62(40.5)	10(6.5)	16(10.5)	6(4)	7(4.6)
	R	99 (64.7)	63 (41.2)	95 (62.1)	55 (36.0)	31 (20.3)	24 (15.7)
	S	45(29.4)	28(18.3)	48(31.2)	83(54.2)	96(62.7)	122(79.)

**Keys:** \* Antimicrobial susceptibility status: **S**, susceptible; **I**, intermediate; **R**, resistant

\*\*AML, Amoxicillin; NOR, Norfloxacin; VA, Vancomycin; CN, Gentamicin; P, Penicillin; CRO, Ceftriaxone

*S. aureus* was resistant to vancomycin and resistance against gentamicin was low (7.7%). Resistance to penicillin, amoxicillin, norfloxacin and ceftriaxone was high among *Klebsiellaspp* with 60.5, 55.3, 47.4 and 39.5% respectively. Antimicrobial resistance of *Proteus* was generally high with 72.2, 66.7, 50.0, 50.0, 33.3 and 27.8 % resistance against amoxicillin, penicillin, ceftriaxone, vancomycin, norfloxacin and gentamicin. Resistance was registered against amoxicillin (62.5%), norfloxacin (56.3%) and penicillin (18.8%) among CNS isolates. However there was no resistance against ceftriaxone, vancomycin and gentamicin. 71.4, 50.0,

35.7 and 28.6% of *Serratia* were resistant to amoxicillin, penicillin, norfloxacin, ceftriaxone and gentamicin. No *Serratia* isolate was resistant to vancomycin. All the 11 isolates of streptococci were resistant to amoxicillin and penicillin, while 72.7, 36.4, 36.4 and 27.3% of the isolates were resistant to ceftriaxone, vancomycin, gentamicin and norfloxacin. Of the 7 isolates of *E. coli* obtained in the study 71.4, 57.1, 57.1, 28.6 and 14.3% were resistant to amoxicillin, penicillin, vancomycin, ceftriaxone and gentamicin. No isolate of *E. coli* was resistant to norfloxacin. Of *Citrobacter* isolated in the study 66.7% were resistant to amoxicillin, penicillin and

vancomycin, and 33.3% were resistant to gentamicin while no resistance was recorded against norfloxacin. All Enterobacter were resistant to amoxicillin and penicillin, and 75.0 and 50.0% were resistant to vancomycin and ceftriaxone. There was no resistance to norfloxacin and gentamicin.

### 3.4 Antimicrobial resistance patterns of isolates

The overall resistance patterns of bacteria isolated from indoor air of selected hospital wards indicate that, 19(12.4%) of the total 153 isolates were susceptible to all tested drugs, while 20 (13.1%) of the total isolates were resistant to only one of the tested antibiotics. One (0.7%) of the total isolates was resistant against all tested antibiotics. Of the total isolates 113 (73.8%) were shows multiple drug resistant).

## 4. Discussion

Resistance to antimicrobial agents is a problem in health care facilities; in hospitals transmission of resistance bacteria is amplified because of the highly susceptible population (WHO, 2002).The isolates showed different degree of susceptibility against tested antimicrobial agents. Gram positive isolates were sensitive to gentamicin (80.3%) following by norfloxacin (63.6%), while they were resistant to amoxicillin (63.6%) and penicillin (57.6%). In agreement with our finding, a study conducted in Nigeria demonstrated that, gram positive isolates were more sensitive to gentamicin (93.3%), while resistance to ceftriaxone was high (71.1%) (Chikere *et al.*, 2008). High level of sensitivity of gram negative bacteria to gentamicin (79.3%) was observed in this study which is in agreement with the findings of Chikere *et al.* (2008). Martin *et al.* (2005) stated that more than 70% of the bacteria that cause hospital-acquired infections are resistant to at least one of the drugs most commonly used to treat these infections, in agreement with this, the present study indicated that 73.8% of the isolates had resistance against two or more of tested antibiotics (multiple drug resistance).The susceptibility patterns of isolates revealed varying degrees of resistance to the antibiotics tested. In the present study most of the isolates 79.7% were susceptible to gentamicin. In line with our finding, a study conducted on antibiogram of clinical isolates from a hospital in Nigeria demonstrated that 93.3% of the isolates were sensitive to gentamicin (Chikere *et al.*, 2008). Resistance of *S. aureus* to vancomycin was 0 %. Similar to our result, studies conducted at Tekur Anbessa University Hospital in Addis Ababa (Gedebou *et al.*, 1988) and at JUSH (Chalachew *et al.*, 2009) recorded very high (100%) sensitivity of *S. aureus* to vancomycin.

## 5. Conclusion and recommendation

### 5.1 Conclusion

The study revealed that there is alarmingly high level of antimicrobial resistance among bacteria isolated from indoor air of the hospital; 86.9% of the total isolates were resistant to 1 or more antibiotics and 73.8% were multidrug resistant. Resistance was especially high to amoxicillin (64.7%) and penicillin (62.1%). From the

result of this study, gentamicin in which 79.7% of isolate were susceptible was the drug of choice for infection caused by hospital indoor air borne bacteria which is causative agents for NIs in the hospital.

### 5.2 Recommendation

Based on the findings of the study the following recommendations are made:

- ✓ Reducing population loads and foot traffic in and out of the wards, especially during time of dressing of wounds.
- ✓ Restricting visitors.
- ✓ Hospital management is advised to periodically assess the quality of indoor air, especially around inpatient wards to identify and minimize/eliminate sources of microbial contamination.
- ✓ Periodic surveillance of drug susceptibility of hospital airborne isolates may contribute to empirical treatment of patients acquiring NIs.
- ✓ Finally, more comprehensive further study is recommended in all wards of the hospital to assess microbial contamination level of indoor air (including fungi and anaerobic bacteria) and to identify the associated factors

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