Bacteria Isolates from Oral Cavities of Malnourished Children at Specialist Hospital, Sokoto, Nigeria

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Abstract

Background

Bacteria Isolated from the Oral Cavities of In-Patient Malnourished Children At Specialist Hospital, Sokoto, Nigeria. Research conducted at Sokoto State Specialist Hospital looked into oral bacteria in malnourished children. Malnutrition stems from nutrient inefficiency, and diet influences health significantly. A microbiological method of isolation involves aseptic oral swabs for examination. Samples are collected by spinning the swab across the mouth for 15–20 seconds. Self-sealing polythene bags transport specimen containers to the lab, ensuring accurate results. Isolation uses nutrient agar, sorbitol, and broth agars. Gram staining, biochemical testing detect nutritious microorganisms on the agar-spread plate. The slide is dried and examined under a microscope with an oil immersion objective lens. With molecular characterization of bacteria isolates from the oral cavity of the patients using PCR.

Results

Haemophilus influenzae, Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Cronobacter condiment, Photorhabdus luminscenes, Klebsiella aeruginosa, Bacillus tequillensis,

Yersinia molderath, and Bacillus megaterium from the patient's oral cavity. Twenty patients' mouths yielded 10 bacterial species. Among bacterial isolates, 25% are Cronobacter condimenti. In oral samples, Klebsiella aeroginosa, Haemophilus influenza, Staphylococcus aureus, Escherichia coli, and Streptococcus pyogenes were 9.1%, 12.5%, and 9.1%, respectively, while Bacillus tequillenses and Yersinia molderath were 4.5%. Klebsiella aeroginosa was found in 15% of patients, Photorhabdus luminscenes in 10%, Haemophilus influenza in 10%, Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Bacillus megaterium, Cronobacter condimenti, and Bacillus tequillensis in 5%. We identified ten bacterial strains based on colony from the morphology, biochemical assays.

Conclusions

Malnutrition affects millions of poor children around the world. It causes short-term disease and death and can affect cognitive function, economic productivity, and reproduction. Food security, healthcare access, and proper oral hygiene and nutrition are needed to combat malnutrition.

KEY WORDS: *Children, Oral cavity, Bacteria, Malnutrition, Isolate, Specialist Hospital and In-patient.*

INTRODUCTION

Malnutrition is a spectrum of disorders of under nutrition states. Although malnutrition occurs globally, obesity is common in developed countries, while under nutrition is in Africa, Asia, and the Americas (World Health Organization, 2021). The World Health Organization (WHO) defines malnutrition as deficiency, excess or imbalance in a person's intake of energy and/or specific nutrients in relation to their requirements (2022). To flourish, early childhood development requires a healthy immune system and a healthy diet. According to a study (Asad and Mushtaq, 2012), child malnutrition continues to be a significant public health concern in many regions of the developing world, particularly in South and Central Asia and sub-Saharan Africa (Stephenson *et al.,* 2020). Malnutrition can be caused by a deficiency in particular nutrients, an excess of another nutrient, or an inability to properly utilize the nutrients consumed. Malnutrition remains the leading cause of illness and death among children worldwide. Sixty percent of the 10.9 million annual deaths of children under five can be attributed to it, and two-thirds of those deaths occur within the first year of life (WHO, 2021). Malnutrition or failure to thrive (FTT) is a clinical syndrome in which the infant or child falls out of the main pattern of growth and is constantly under the 3% curve for height and weight or with more than 2 standard deviations below mean height and weight (Malek and Sharifiyan, 1999). Nearly 100 million children under the age of five are suffering from protein-energy malnutrition (PEM) or failure to thrive (FTT) worldwide (Reshad, 1991). Malnutrition in childhood causes delay in body growth, short stature and disturbances in the mental development of children, and is associated with frequent infections and resistance to treatment. In addition to the physical growth retardation in children with malnutrition, the prevalence mental disorders, lack of academic achievement and reduced productivity are very common and these children cannot reach their mental and physical abilities later in life (Berkman *et al,.* 2020) According to the United Nations International Children's Emergency Fund (UNICEF)

statistics, 11% of Iranian children suffer from moderate and severe underweight, and 15% suffer from moderate and severe short stature (Gheyraie, 2002)

Childhood under nutrition encompasses a spectrum of nutritional disorders, ranging from underweight and wasting to stunted growth (Wagnew *et al.,* 2018). Wasted (low weight-for-height) malnutrition is caused by a recent failure of nutrition (e.g., lack of food) or a current infection that causes weight loss, such as diarrhoea. Underweight (low weight for age) is an all-encompassing indicator of wasting and malnutrition (low height for age). Weight-for-height -3Z scores of the median WHO growth guidelines, a mid-upper arm circumference (MUAC) of 115 mm, obvious extreme wasting, and the presence of nutritional edema are diagnostic criteria for severe acute malnutrition. Guesh *et al.* (2018) Undernourishment, obesity, and other NCDs related to diet account for fifty percent of the additional burden of malnutrition. There are four primary signs of malnutrition: wasting, stunting, being underweight, and a deficiency in specific micronutrients. Underweight (low weight-for-age) is a comprehensive index of wasting and stunting. Wasted (low weight-for-height) is acute malnutrition due to a recent failure of nutrition (e.g., lack of food) or a recent infection like diarrhoea that causes weight loss (low height-for-age). Diagnostic criteria for severe acute malnutrition include weight-for-height -3Z scores of the median WHO growth standard, a mid-upper-arm circumference (MUAC) of 115 mm, obvious severe wasting, and the presence of nutritional edoema (Fassikaw *et al.,* 2022). Leanness relative to height is a defining characteristic of wasting. It is a common symptom of rapid and recent weight loss, though the condition can persist for a considerable amount of time. This condition frequently develops when a patient has not eaten enough nutritious food or has been ill frequently enough for an extended period of time. Child wasting increases the likelihood of death if not addressed. Stunting is defined by a low height in relation to age. These conditions are due to inadequate feeding and/or care during infancy and/or childhood. Chronic or recurrent micronutrient deficiencies are frequently associated with low socioeconomic status, pregnancy complications, frequent illness, and/or inadequate food availability.

The oral microbiome, mainly comprising bacteria which have developed resistance to the human immune system, has been known to impact the host for its own benefit, as seen with dental cavities. The environment present in the human mouth allows the growth of characteristic microorganisms found there. It provides a source of water and nutrients, as well as a moderate temperature (Sherwood *et al.,*2013). Resident microbes of the mouth adhere to the teeth and gums to resist mechanical flushing from the mouth to stomach where acid-sensitive microbes are destroyed by hydrochloric acid (Wang *et al.,* 2014).

Anaerobic bacteria in the oral cavity include: *Actinomyces*, *Arachnia*, *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Fusobacterium*, *Lactobacillus*, *Leptotrichia*, *Peptococcus*, *Peptostreptococcus*, *Propionibacterium*, *Selenomonas*, *Treponema*, and *Veillonella*. (Sutter 1984). In addition, there are also a number of fungi found in the oral cavity, including: *Candida, Cladosporium, Aspergillus, Fusarium, Glomus, Alternaria, Penicillium, and Cryptococcus* (Cui *et al.,* 2013). The oral cavity of a new-born baby does not contain bacteria but rapidly becomes colonized with bacteria such as *Streptococcus salivarius*. With the appearance of the teeth during the first year colonization by *Streptococcus mutans* and *Streptococcus sanguinis* occurs as these organisms colonise the dental surface and gingiva. Other strains of streptococci adhere strongly to the gums and cheeks but not to the teeth. The gingival crevice area (supporting structures of the teeth) provides a habitat

for a variety of anaerobic species. Bacteroides and spirochetes colonize the mouth around puberty (Rogers, 2008). Of particular interest in this study is the bacteria in the oral cavity of malnourished children.

Oral microbiology is the study of the microorganisms (microbiota) of the oral cavity and their interactions between oral microorganisms or with the host (Schwiertz, 2016). The environment present in the human mouth is suited to the growth of characteristic microorganisms found there. It provides a source of water and nutrients, as well as a moderate temperature (Sherwood *et al.,*2013). Resident microbes of the mouth adhere to the teeth and gums to resist mechanical flushing from the mouth to stomach where acid-sensitive microbes are destroyed by hydrochloric acid (Wang *et al.,* 2014). Bacteria accumulate on both the hard and soft oral tissues in biofilms. Bacterial adhesion is particularly important for oral bacteria.

Oral bacteria have evolved mechanisms to sense their environment and evade or modify the host. Bacteria occupy the ecological niche provided by both the tooth surface and mucosal epithelium. (Rogers, 2008). Factors of note that have been found to affect the microbial colonization of the oral cavity include the pH, oxygen concentration and its availability at specific oral surfaces, mechanical forces acting upon oral surfaces, salivary and fluid flow through the oral cavity, and age (Lamont *et al.,*2014). However, a highly efficient innate host defense system constantly monitors the bacterial colonization and prevents bacterial invasion of local tissues. A dynamic equilibrium exists between dental plaque bacteria and the innate host defense system (Rogers, 2008). Additionally, research has correlated poor oral health and the resulting ability of the oral microbiota to invade the body to affect cardiac health as well as cognitive function (Noble *et al.,* 2013). Paster and colleagues (Colombo *et al.,* 2009) developed the HOMIM (Human Oral Microbe Identification Microarray) platform but this 16S rRNA gene array is somewhat more global as it aims at simultaneous detecting the most prevalent oral bacterial species, without taking into account any particular disease. Initially intended for the study of bacterial diversity in oral samples, the approach was extrapolated to the study of various microbiota associated either with medical (intestinal or skin flora), environmental (soil, sludge, wastewaters, etc.), or food industry samples; and can be used as a faster and cheaper alternative to cloning sequencing of the ribosomal gene.

METHODOLOGY

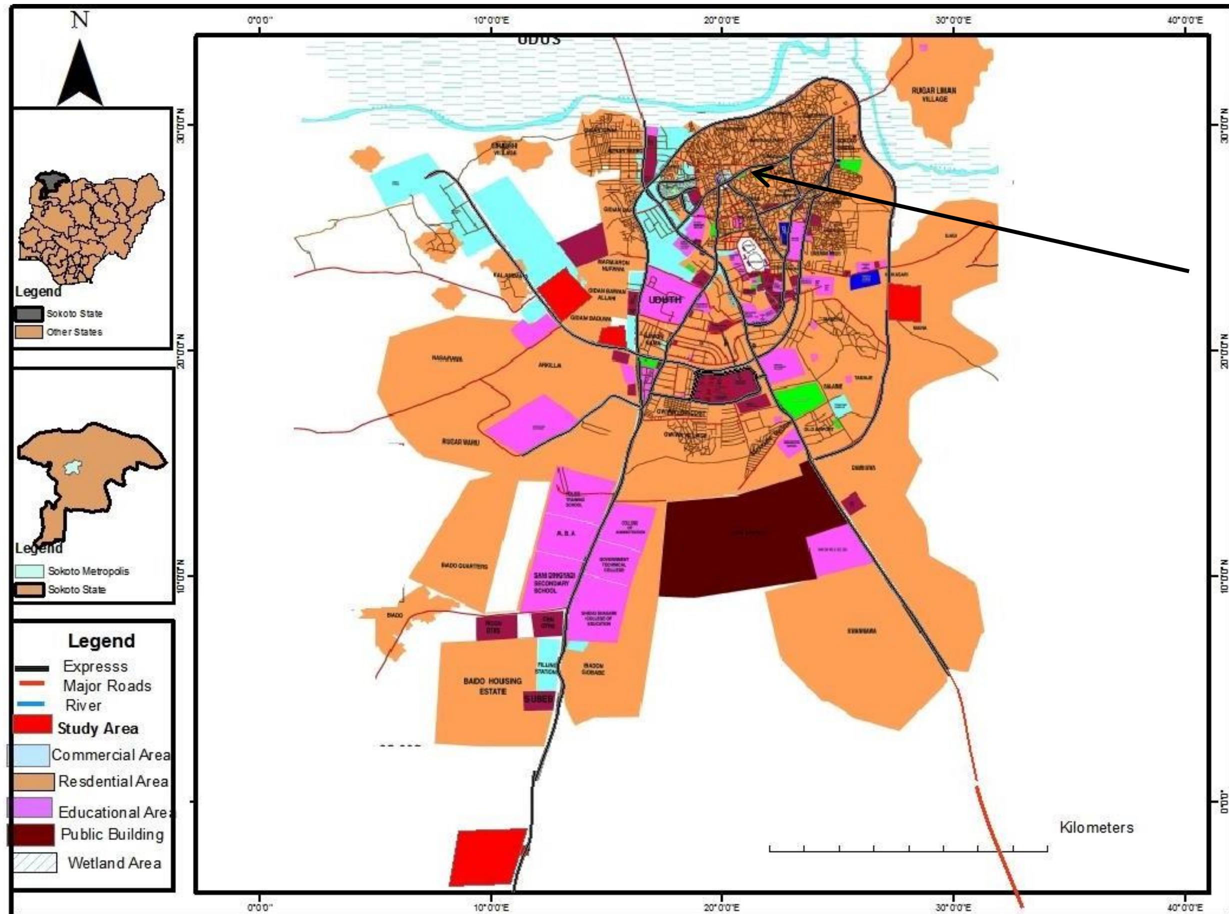
Sokoto, one amongst Nigeria's 36 states, is located in the nation's northwest, adjacent to the independent nation of Niger. Sokoto, the capital and largest city of Nigeria, is situated near the confluence of the Sokoto and Rima rivers. Sokoto derives its name from the Arabic word for "market," Sakkwato. It is also known as Birnin Shehu da Bello and Sakkwato (Sokoto, Capital of Shehu and Bello). The city is home to a large Muslim population and the caliphate's headquarters, which are located in the Islamic educational complex of Nigeria. Since its incorporation as a province of the British protectorate of Nigeria in 1900, Sokoto has covered the northwest corner of Nigeria since its formation in 1976 from the division of North-Western State into Sokoto and Niger States (Figure 1). The Sultan of the caliphate provides Nigeria's Muslims with spiritual leadership.

The Sokoto Specialist Hospital is a Public hospital own by the government, located at Rijiya A, Sokoto South Local Government, Sokoto State. It was established on 1986-01-01, and operates on 24hrs basis.

The Sokoto Specialist Hospital is not applicable hospital by the Nigeria Ministry of Health, with

facility code 33/17/1/2/1/0001 and registered as Secondary Health Care Centre. The Sokoto Specialist Hospital is open on Monday, Tuesday, Wednesday, Thursday, Friday, Saturday, Sunday, and operates on 24 hours basis with a bed capacity of over 700 bed spaces. It is registered as a Secondary Health Care Centre.

Fig 3.1: The Administrative Map of Sokoto State in Nigeria showing location of Specialist Hospital, Sokoto.



Limitations of the Calculated Sample Size

The sample size calculation in the preceding formula (effect size and standard deviation). The number of participants in the research study was predetermined and, if at all possible, will not be changed. Some logistical factors, like how the government works and how much money is available, can affect the estimation of sample size.

Consent

Patients will be given informed consent for specimen collection and, as part of the process, as attached in appendix 1, 2, 3 and 4.

- Inform the patient of the reason for specimen collection;
- Explain the procedure;
- Assess the patient's understanding.

Samples collection

Oral swab samples from the malnourished patientin specialist Hospital, sokoto state were taken using aseptic techniques for microbiology analysis (Cheesbrourgh, 2006). Surveys were given to hospitalised patients who had agreed to donate samples.

The patient was instructed to forego eating, drinking, brushing their teeth, and using mouthwash for at least 30 minutes prior to the collection of the swab. A Puritan PurFlock Ultra (model number 25-3606-U) was used to carry out the rapid repeat swabbing analysis. Participants in the study who are in-patients were instructed to stick out their tongues, and a sterile swab was used to run along the length and width of the front two-thirds of the subject's tongue. The swab shafts will only very slightly bend as a result of the pressure. The samples were taken while the swab was rotating moved around the oral cavity for fifteen to twenty seconds (Liabeya et al., 2019). Each patient has five millilitres of blood taken from them.

Inclusion and Exclusion Criteria

Inclusion

Inclusion criteria were children of either gender under six years who were referred to the paediatrics clinic of the hospital because of malnutrition and also various complaints that consequently required hospitalization..

Exclusion

Exclusion criteria were children with congenital malformations, chronic diseases, and any systematic disorders. The patients were evaluated and selected by together with a paediatrician. After taking informed consent from the parents or guardians of children, they were asked questions based on the aim of the study

Preparation of Media

Following is a description of how to create some of the most fundamental microbiology media. Unless otherwise specified, the pH was 7 and the sterilisation temperature will be 121 degrees Celsius for 15 minutes.

Nutrient Agar

Suspend 1 litre of distilled water dissolves 28 g of nutrient agar powder. Boil to dissolve. Sterilized and dispensing as needed.

Nutrient Broth

The powdered nutrient broth will weigh 13 grammes, and one litre of distilled water will be used to dissolve it. Reconstitute and sterilise before dispensing as needed.

Isolation and Characterization of Bacteria isolates

Spread plate method using nutrient agar were used to isolate the bacteria; gram staining and biochemical test were used to identify the species of bacteria isolated (Cheeseborough, 2006).

Isolation of bacteria

The specimens were collected using a swab stick in the oral cavity under good lighting. To collect a surface sample, the swab stick was firmly inserted into the oral cavity. The swab was moistened

before returning it to the tube, with the media at the bottom of the tube. Label and transport the swab to the laboratory with a patient-specific identifier. The collected specimen arrived at the laboratory within one hour to be plated; if it takes longer, it will be refrigerated (Cheeseborough, 2006).

Gram staining
A smear of the bacterial culture was prepared by placing a drop of sterile water on a clean slide, spreading a bacterial colony on it, and allowing it to air-dry. The reverse side of the slide was repeatedly passed over a flame. The smear was saturated with crystal violet for sixty seconds before being rapidly rinsed with water. The smear was stained with Gram's iodine for 60 seconds, the iodine was poured off, and the smear was held in 95% ethanol until the crystal violet stain disappeared for 10 to 15 seconds before being rinsed with water. Safranin (a counterstain) was applied to the stain for 30 seconds. The slide was held in water, blotted dry, and viewed with an oil immersion objective lens (x100) under a microscope (Cheesbrough 2006).

Biochemical Tests
Based on the variations in the biochemical activities of various bacteria, biochemical tests are used to identify the species of bacteria. The physiology of bacteria varies depending on the type of organism. Indole, motility, the starch test, the MR-VP, urea, oxidase, citrate, coagulation, and the spore-forming test are some of the tests included in this one (Cheeseborough, 2006).

Indole test
Sterile test tubes containing 4 mL of tryptophan broth inoculate the tube aseptically with the growth from 18 to 24 hours of culture, and then incubate at 37 degrees Celsius for 24-28 hours. There will be an addition of 0.5 mL of Kovac's reagent to the broth culture. Observe whether the ring is present or absent (Cheesbrough 2006).

Motility
After picking a well-isolated colony, the medium will be pierced with a sterile needle to within 1 cm of the bottom of the tube. The medium was incubated for 24 hours at 37 °C. Upon completion of the incubation period, a red, turbid area extending from the inoculation line will indicate a positive result. A negative result was indicated by a red growth along the inoculation line but no other indication of a negative result (Cheesbrough, 2006; Jarell and Mc Bride, 2008).

Methyl red reaction
Zero-point one millilitre (0.1 ml) of MR-VP broth was inoculated with the test organism and incubated for 24-48 hours at 35 degrees Celsius. Five drops of methyl red are added to five millilitres of culture. The red colour indicated a positive reaction, whereas the yellow colour indicated a negative reaction (Cheesbrough 2006).

Voges Proskauer
In a test tube, 5 ml of MR-VP broth was inoculated with the test organism and incubated for 48 hours at 35 °C. Then, 0.6 ml of an alpha naphthol solution and 0.2 ml of a 40% potassium hydroxide solution were added to 1 ml of the culture, which was stored for two to four hours. Red coloration indicated a positive reaction, whereas no colour change indicated a negative reaction (Cheesbrough 2006).

Urease test
The colony from a preserved isolate was streaked onto urea agar medium with a sterile wire loop and incubated at 37°C for 48 hours. The appearance of a vibrant purple-pink or red colour indicated favourable results (Cheesbrough 2006).

Oxidase test
In a sterile Petri dish, a piece of filter paper was placed, and two drops of freshly prepared oxidase reagent (redox indicator) were added. A colony of the organism was scraped onto the filter paper using a sterile wire loop. The appearance of a blue-purple hue within a few seconds indicated positive results, while a purple hue indicated oxidase negativity (Cheesbrough 2006).

Citrate utilization
The test organism was inoculated onto a slant containing Simmons's citrate agar and incubated for 48 hours at 35 degrees Celsius. Growth and colour change from blue to blue-green indicated a positive response, whereas the absence of colour change indicated a negative response (Cheesbrough 2006).

Coagulase test
On a slide, a drop of physiological saline was placed. In the drop of saline, a colony of the test organism was emulsified to create a thick suspension. Then, a drop of plasma that had been defibrillated was added to the suspension and gently mixed. Within 10 seconds, clumping of the organism will be observed, indicating a positive result (Cheesbrough 2006).

Triple sugar iron test (TSI)
The TSI medium is composed of three sugars: glucose, sucrose, and lactose. The inoculum was streaked on the surface of the agar slants with wire-loop, and the caps were tied loosely and incubated for 24 hours at 37°C. After the incubation period, the tube was observed for the formation of hydrogen sulphide, which was indicated by the blackening of the slant bottle, sugar fermentation (for glucose, lactose, and sucrose), and gas production, which was indicated by the yellowing of the slant bottle (Cheesbrough 2006).

RESULT

Ten bacterial strains were found in the oral cavity of the malnourished children includes: *Haemophilus influenzae, Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Cronobacter condimenti, Photorhabdus luminscenes, Klebsiella aeroginosa, Bacillus tequillensis, Yersinia molderath, and Bacillus megaterium* (Table 1). Fig 2; is a graphical representation of the bacterial isolates, which serves as an indicator of occurence. *Cronobacter condimenti* had the highest occurrence in oral cavity of 25%. *Bacillus tequillenses, Yersinia molderath, and Bacillus megaterium* all had 4.5% in oral samples, while the occurence for *Klebsiella aeroginosa, Haemophilus influenza, Staphylococcus aureus, Escherichia coli,* and *Streptococcus pyogenes* had 9.1%, 12.5%, and 9.1%, respectively. Among the bacteria found in patients, *Klebsiella aeroginosa* (15%), *Photorhabdus luminscenes* (10%), *Haemophilus influenza* (10%), *Staphylococcus aureus* (10%), *Escherichia coli* (10%), *Streptococcus* pyogenes (10%), *Bacillus megaterium* (5%), *Cronobacter condimenti* (5%), *and Bacillus tequillensis (5%)* (Table 1)

Haemophilus influenzae

Morphology Gram-negative, 0.3-0.5 / 0.5-3.0 pm, rods or coccobacilli, nonmotile, but fresh clinical isolates express peritrichous pili with hemagglutinating properties which are lost after

subcultivations. Fimbrial structures have been demonstrated in otitis media isolates of noncapsular strains. Many strains produce capsules.

Cultural characteristics Colonies on chocolate agar are smooth, low, convex, grayish, translucent, 0.5-1.0 mm in diameter in 24h and 1-1.5 mm in 48h. Capsulated strains produce larger and more mucoid colonies (1-3 mm) with tendency to coalesce. On transparent agar media, colonies of capsulated strains show iridescence when examined under obliquely transmitted light. Morphologically atypical strains produce more granular colonies. Hemolysis is negative. Aerobic, facultatively anaerobic, require X-factor and V-factor for growth. CO_2 is not required. Optimum temperature 35-37 °C. Growth on Mac Conkey agar is negative.

Biochemical characters Positive results for nitrates reduction, oxidase, alkaline phosphatase, catalase, acid production from: D(+) glucose (without gas), D(+) xylose, D(+) galactose & maltose.

Negative results for H25 production, arginine dehydrolase, ONPG, alpha-glucosidase, acid production from: glycerol, xylitol, L(+) arabinose, dulcitol, meso-inositol, mannitol, D(-) sorbitol, D(-) fructose, D(+) mannose, L(+) rhamnose, L(-) sorbose, cellobiose, lactose, D(+) melibiose, sucrose (saccharose), trehalose, raffinose, inulin & salicin. Variable results for urease (1), lysine decarboxylase, ornithine decarboxylase (2> & indole production (3).

(1) urease test is positive for biovars 1.11,111,IV and negative for biovars V.VI,VII. (2) Ornithine decarboxylase is positive for biovars I. IV. V (H. influenzae subsp. meningitidis) & VI and negative for biovars 11.III.V11 (H. influenzae subsp. influenzae). (3) Indole test is positive for biovars 1.II, V.V11 and negative for biovars III,IV,VI.

Staphylococcus aureus

Morphology

Gram-positive cocci, 0,8-1 pm, nonmotile, nonsporing. Occur in clusters resembling grapes (staphylo-).

Cultural characteristics

Subsp. aureus: after 48h of incubation colonies are circular, opaque, yellowish, 0.5-2 mm diameter, S-type, often hemolytic on blood agar. May produce capsules. Encapsulated strains usually produce smaller and more convex colonies. Temperature range for growth 10-45 °C; optimum 30-37 °C. Facultatively anaerobic.

Subsp. anaerobius: colonies on blood agar are white (non-pigmented), opaque, glistening, and entire, smooth, and convex; 1-3 mm in diameter after 2 days incubation. Temperature range for optimal growth 30-40 °C. No growth at 20 or 45 °C.

Both subspecies grow good in medium containing 10% NaCI; poor/none at 15% NaCI. Grow on Trypticase Soy Agar ± 5% sheep blood, Mueller Hinton agar, Dorset with egg (for subsp. anaerobius), Chapman (selective medium with 75 g/I NaCI & mannitol), Baird-Parker agar (selective medium with lithium chloride and potassium tellurite).

Biochemical characters Positive results for alkaline phosphatase, coagulase, DN-ase, FDP-aldolase class 1, heat-stable nuclease, hyaluronidase, urease, acid production from fructose, and sucrose.

Negative results for beta-galactosidase, beta-glucuronidase, oxidase, acid production from arabinose, cellobiose, melezitose, raffinose, salicin, xylitol, and xylose. Ecology Isolated from human & animal skin, ears and mucous membranes; ubiquitous. Novobiocin sensible.

Escherichia coli

Morphology Gram-negative, 0.5-1.5 x 2.0-6.0 pm, straight, bacilli or coccobacilli. Non-spore-forming. Motile by peritrichous flagella or non-motile.

Cultural characteristics Colonies on Nutrient agar may be smooth (S type), low convex, shiny surface, entire edge, gray. R type or mucoid forms may occur. Colonies of E. hermanii are yellow -pigmented. Aerobic, facultatively anaerobic. Optimum growth temperature 37 °C. Grow readily on simple nutrient media like Nutrient agar or nutrient broth. For haemolysis use Trypticase Soy Agar + 5% sheep blood. Selective media: GEAM / Levine - black colonies with metallic surface, Mac Conkey agar - red colonies, Rambach agar -blue-green colonies, Leifson - red colonies, Istrati-Meitert - yellow colonies.

Biochemical characters Glucose and other carbohydrates are fermented with the production of pyruvate, which is converted into lactic, acetic and formic acids. Part of the formic acid is split into CO2 and Hz.

Positive results for catalase, nitrate reduction, acid production from: L-arabinose, D-mannitol, D-mannose and trehalose.

Negative results for oxidase, H25 production, urease, Voges-Proskauer reaction, DN-ase, lipase, gelatinase, citrate utilization, phenylalanine and acid production from inositol (unknown for E. ruysiae).

Streptococcus pyogenes

Morphology Gram-positive, spherical or ovoid cells, 0.5-1 pm in diameter. Grouped in pairs or short to moderate chains in clinical materials; long chains in broth cultures. Some strains form a capsule of hyaluronic acid.

Cultural characteristics Colonies may look mucoid, glossy or matt. Beta-hemolytic on blood agar (streptolysin 0). Facultative anaerobes, optimal temperature 37.0 °C, no growth at 10 or 45 °C. Nutritionally fastidious, especially upon primary isolation. C, No growth in the presence of 6.5% NaCI, at pH 9.6, or in the presence of 40% bile.

Biochemical characters Fermentative metabolism, the final pH in glucose broth is 4.8-6.0. Positive results for alkaline phosphatase. Arginine hydrolysis, pyrrolidonyl arylamidase, acid production from: N-acetylglucosamine, fructose, glucose, galactose, lactose, maltose, salicin, sucrose, methyl D-glucoside & trehalose.

Negative results for alpha- or beta-galactosidase, beta-glucosidase, N-acetyl-beta-glucosaminidase. Glycyl-tryptophan arylamidase: hippurate hydrolysis, Voges-Proskauer

reaction, acid production from: adonitol, amygdalin, arbutin, arabinose, arabitol, dulcitol, cyclodextrin, erythritol. Gluconate, inulin: melibiose, methyl D-mannoside, mannitol, melezitose, raffinose, ribose, sorbitol, sorbose. tagatose. methyl D-xyloside & xylose.

Variable results for esculin hydrolysis and beta-glucuronidase.

Cronobacter condimenti

Morphology Gram-negative rods. Non-motile. Non-spore-forming.

Cultural characteristics Colonies on TSA incubated at 37 °C for 24 hours are 2-3 mm in diameter, opaque: circular and yellow. Grows on MacConkey agar. In TSB, grows at 45 °C (optimum 37 °C), but not at 5 °C. No haemolysis is observed on sheep blood agar at 37 °C. Facultatively anaerobic.

Biochemical characters Positive results for arginine dihydrolase, catalase, citrate utilization, DNase (delayed), alpha-glucosidase, and beta-galactosidase, gelatin hydrolysis: indole production, malonate utilization, nitrate reduction, ornithine decarboxylase, Voges-Proskauer test: acid production from L-arabinose, amygdalin, cellobiose, glucose (without gas production), inositol, mannitol, melibiose, rhamnose, and saccharose. Can ferment/oxidize L-arabinose, arbutin, cellobiose, esculin, D-fructose: 0- and L-fucose, galactose, D-glucose, 10-methyl alpha-D-glucopyranoside, glycerol, beta-gentiobiose, galacturonic acid, myo-inositol, lactose, maltose, D-mannose, D-mannitol, melibiose, N-acetylglucosamine, raffinose, L-rhamnose, ribose, salicin, sucrose, trehalose, and D-xylose

Negative results for H2S production, lysine decarboxylase, methyl red test: oxidase, urease, acid production from adonitol, D-sorbitol and 5-ketogluconate. No fermentation/oxidation of D- and L-arabitol, D-arabinose, dulcitol, gluconate, 2- and 5-ketogluconate, inositol, lactulose, maltitol, melezitose, putrescine, cis- or transaconitate, 4-aminobutyrate, palatinose, D-erythritol, adonitol, methyl beta-D-xyloside. L-sorbose, sorbitol, methyl alpha-D-glucoside, inulin, glycogen: xylitol, D-lyxose, D-tagatose, and turanose.

Photorhabdus luminescens

Morphology Gram-negative rods, 2-6 x 0-5-1.4 pm. Motile by peritrichous flagella. Produce a faint glow. The suggested mode of light production is that cell uses the luciferase pathway as a terminal oxidase, allowing it to continue aerobic metabolism in low oxygen conditions.

Cultural characteristics Polimorph, yellow, orange, or brown colonies. Optimum growth temperature 28 °C. Maximum temperature for growth in nutrient broth is 38-39 °C. Bioluminescent. Annular hemolysis observed on sheep-blood agar and, in some strains, on horse-blood agar. Spontaneous phase shift occurs in subcultures inducing the appearance of phase II clones. Bioluminescence is more intense in phase I. Facultatively anaerobic.

Biochemical characters Positive results for catalase, esculin hydrolysis, lecithinase (egg yolk agar), Tween 20 and 80, acid production from: fructose, glucose (without gas production), glycerol, N-acetyl-glucosamine, maltose(s), D-mannose(s/), ribose and trehalose(s) (most strains).

Negative results for arginine dihydrolase, DNase(s/), lysine decarboxylase, H2S production, growth on KCN media, methyl red(s/), nitrate reduction, omithine decarboxylase, oxidase, ONPG,

phenylalanine deaminase, acid production from: amygdalin, adonitol(sO, L-arabinose, cellobiose, dulcitol, alpha-methyl-D-glucoside(d), lactose(s1), melibiose, L-rhamnose(s/), raffinose, salicin(d), D-sorbitol, sucrose, and D-xylose(s1).

Legend: (8) data available only for subsp. luminescens.\ Ecology The natural habitat is in the intestinal lumen of entomopathogenic nematodes of Heterorhabditis bacteriophora and H. mexicana and insects infected by these nematodes.

Genus Klebsiella

Morphology Gram-negative, straight, non-motile, capsulated, 0.3-1.0 pm x 0.6-6 pm bacilli. K. oxytoca strains produce a dark brown pigment when growth on media containing gluconate and ferric citrate.

Cultural characteristics Glistening moist colonies of varying degrees of stikiness (M—type colonies). Klebsiella granulomatis grows intracellulaly only; it has not been cultured. No special requirements. Facultatively anaerobic, optimum growth temperature 35-37 °C. Grows on: Nutrient agar/broth, Trypticase Soy Agar ± 5% sheep blood, Mac Conkey. Mueller-Hinton agar. K. granulomatis grow in the yolk sac of developing embryo or in cell cultures (fresh mononuclear cells or Hep-2 cell line.

Biochemical characters Positive results for catalase, nitrate reduction (except K. singaporensis), esculin hydrolysis (most strains), acid production from: L-arabinose, D-adonitol, cellobiose, glucose, mannitol, melibiose, maltose, D-mannose, raffinose, salicin, trehalose and D-xylose.

Negative results for oxidase, arginine dihydrolase, H_2S production, phenylalanine deaminase, gelatin hydrolysis, DN-ase and lipase.

K. granulomatis doesn't grow in bacteriological media. K. africana is biochemically similar to K. pneumoniae complex members and can be differentiated by the inability to metabolize D-arabitol. K. grimontii is distinguishable from K. oxytoca and K. michiganensis by the inability of most strains to ferment melezitose. K. michiganesis is distinguishable from K. oxytoca by no urease production, no utilization of putrescine, and no pectate degradation. K. quasipneumoniae is difficult to be differentiated from K. pneumoniae using biochemical tests. K. quasipneumoniae can utilize tricarballylic acid and may ferment L-sorbose. K. pasteurii is negative for lysine decarboxylation. K. spallanzanii is negative for Voges-Proskauer test and unable to use L-proline. K. variicola - can only be genetically differentiated from K. pneumoniae strains; does not ferment adonitol.

Bacillus tequilensis

Morphology

Gram-positive, motile rods, 0.9 x 4.0 pm, single cells. Produce central endospores in unswollen sporangia.

Cultural characteristics

Colonies are smooth: circular, yellowish: up to 4 mm in diameter after 1 days at 37 °C on tryptone soy agar. Nonhemolytic on 5% sheep blood agar. Aerobic. No anaerobic growth. Growth temperature 25 - 50 °C, pH 5.5 - 8.

Biochemical characters

Positive results for oxidase, catalase, arginine dihydrolase, beta-galactosidase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, hydrolysis of gelatin, hydrolysis of casein, hydrolysis of starch: indole production, nitrate reduction, tryptophan deaminase, Voges-Proskauer test, acid production from: L- or D-arabinose, adonitol, L- or D-arabitol, amygdalin, N-acetyl-D-glucosamine, arbutin, cellobiose, dulcitol, fructose, L- or D-fucose, galactose, beta-gentiobiose, D-glucose, glycerol, glycogen, meso-inositol, inulin, lactose, lyxose, maltose: D-mannitol, D-mannose, melibiose, raffinose, rhamnose, ribose, sorbitol, salicin, sorbose, sucrose, starch, trehalose, xylitol, L- or D-xylose.

Negative results for hydrolysis of urea, acid production from: erythritol, gluconate. 2- or 5-ketogluconate, melezitose & methyl beta-xyloside.

Biochemically is very similar to B. subtilis from which can be differentiated by positive arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and acid production from rhamnose.

Genus Yersinia

Morphology

Gram-negative, bipolar, 1.0-3.0 x 0.5-0.8 pm rods. Motile in vitro, but nonmotile in vivo. Non-motile at 37 °C, but motile with 2-15 peritrichous flagella when grown below 30 °C, except for Yersinia pestis, which is always nonmotile. No capsule & no spores are produced.

Cultural characteristics

Small, gray-white, opaque, irregular colonies, 0.1 to 1.0 mm in diameter after 24 hours incubation. Y. pestis strains do not produce pigment but adsorb large amounts of exogenous hemin that cause formation of apparently pigmented colonies at 26 °C (not at 37 °C). Facultatively anaerobic, optimum growth temperature 28 °C. Grow very slowly at 4 °C (1-3 weeks). Media: Nutrient Agar or Nutrient Broth, Trypticase Soy Agar ± 5% sheep blood, Mac Conkey - lactose negative colonies.

Biochemical characters some tests results may be negative at 37 °C; optimum temperature is 28 °C.

Positive results for catalase, methyl red (most strains), nitrates reduction (most strains), acid production from glucose (usually without gas), D-mannitol, D-mannose and trehalose.

Negative results for oxidase, arginine dihydrolase, lysine decarboxylase, DN-ase, H2S production, Voges-Proskauer reaction, phenylalanine deaminase, gelatin hydrolysis, growth on KCN medium, citrate utilization, malonate utilization, acid production from: lactose (most of strains), meso-inositol, dulcitol, mucate, erythritol and adonitol. Ecology Isolated mostly from water, some from fish, snails, oysters, frogs, pasteurized milk. Also isolated from humans (feces, sputum, blood, urine, absceses, and lymph nodes), animals (rodents, swine, cattle, birds), soil and foods (milk, meat).

Bacillus megaterium

Morphology

Gram-positive, 2.0-5.0 x 1.2-1.5 pm, motile, peritrichous flagella. Spores are ellipsoidal or spherical, central, paracentral or subterminal; not swelling the sporangia. Capsule might be present Cells grown on glucose agar produce large amounts of storage material, giving a vacuolate or foamy appearance.

Cultural characteristics

Colonies are round to irregular, with entire to undulate margins. May become yellow and then brown or black after prolonged incubation. Hemolytic. Aerobic, do not grow anaerobically. Growth temperature from 3-20 °C to 35-45 °C. Optimum temperature is 30 °C. The temperature range of a water isolate from an Antarctic geothermal island was 17-63 °C, with an optimum of 60 °C. On nutrient agar grow heaped and non-spreading, glossy, sometimes slightly rugose;mucoid on glucose agar. Aged cultures may become yellow, brown or black. Growth active at pH 5.7-7.0 Grow in7 % NaCI. NaCI, allantoin or urate are not required for growth.

Biochemical characters

Positive results for catalase, hydrolysis of starch, utilization of citrate, hydrolysis of esculin, beta-galactosidase, phenylalanine deamination, hydrolysis of gelatin, hydrolysis of casein, acid production from: L-arabinose, N-acetyl-D-glucosamine, amygdalin, arbutin, D-cellobiose, D-fructose, galactose, beta-gentibiose, meso-inositol, inulin, lactose, glycogen, glucose, glycerol, maltose, D-mannitol, D-melibiose, D-raffinose, ribose, salicin, starch, sucrose, trehalose, and D-xylose.

Negative results for Voges-Proskauer, indole production, arginine dihydrolase, lysine decarboxylase, omithine decarboxylase, tryptophan deaminase, hydrolysis of urea, egg yolk reaction, acid production from: methyl beta-xyloside, D-arabinose, adonitol, L-arabitol, dulcitol, erythritol, D- or L-fucose, 2- or 5-ketogluconate, lyxose, D-mannose, rhamnose, and sorbose.

Variable results for oxidase, degradation of tyrosine, nitrate reduction, acid production from: D-arabitol, gluconate, D-melezitose, sorbitol, and xylitol.

Variable results for oxidase, degradation of tyrosine, nitrate reduction, acid production from: D-arabitol, gluconate, D-melezitose, sorbitol, and xylitol.

DISCUSSION

The human oral cavity can be thought of as a microcosm, with diverse ecological niches such as the anterior and posterior surfaces on the tongue, the mucosal epidermal layer of the firm mouth, the palate's soft part, and supra-gingival debris on tooth surfaces. These niches are occupied by a varied variety of microbes, including fungi, viruses, and bacteria (Alghamdi, 2022). According to Chen et al. (2010), 1100 different taxa were found and catalogued in the Man Oral Microbiome Database. The buccal cavity is home to a varied microbial population dominated by Firmicutes, bacterial genera, Proteobacteria, Spirochaetes, and Fusobacteria, despite a limited representation of other phyla (Bik et al., 2010). Figure 2 to 6 depicts this composition. Certain bacteria in this category provide significant benefits, while others have the potential to cause severe infections.

Certain bacteria have the ability to go from a beneficial to a harmful lifestyle, resulting in severe oral cavity infections (Ahn et al., 2012). These bacteria have developed an intimate

relationship with human body over the period of time and represents the single most abundant microflora in human microbiome structure (Rahman et al., 2015). As a result, these microbes exhibit opportunistic traits.

CONCLUSIONS

The results of this investigation confirmed that the oral cavity of malnourished patients, and particularly in-patients could harbor microorganisms, and these bacteria are frequently implicated in multiresistant, systemic, oral or nosocomial infections. Consequently, in this study, we were able to isolate and identify several oral bacterial strains which belonged to the species.

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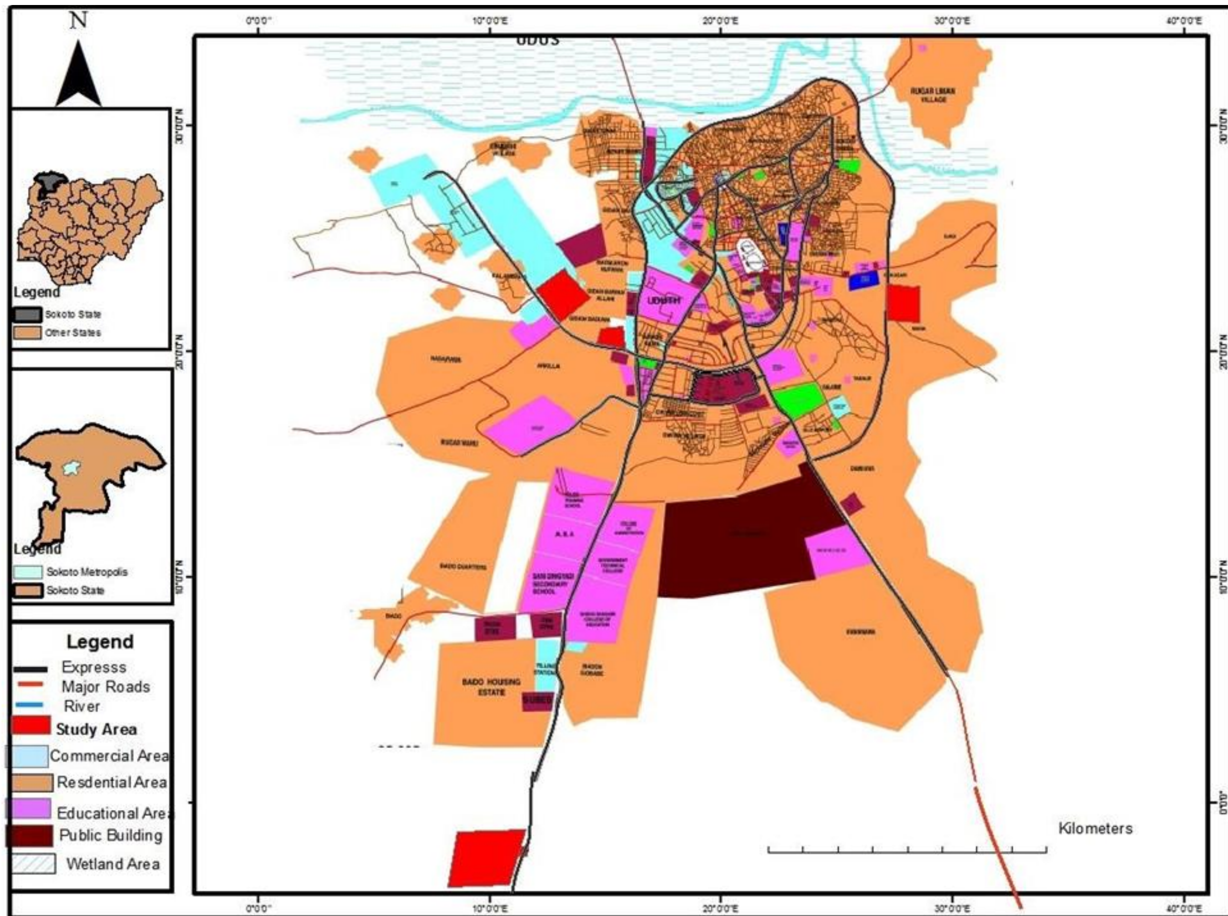

Fig 1: The Administrative Map of Sokoto State in Nigeria showing location of Specialist

TABLE : 1 BIOCCHEMICAL REACTION OF BACTERIA ISOLATED FROM ORAL CAVITY OF MALNOURISHED CHILD

S/N	Shape	Grxm	CIT	MR	VP	GLU	LAC	SUC	FRUC	H2S	GAS	URE	CAT	BACTERIA
1	Rod	+	+	-	+	+	+	+	+	-	+	-	+	Klebsiella aeroginosa
2	Rod	-	+	+	+	+	+	+	+	-	+	-	+	Bacillus tequilensis
3	Rod	-	-	-	+	+	-	-	-	-	-	-	-	Yersinia molderatti
4	Rod	-	-	+	-	-	+	+	+	-	+	-	-	Bacillus megaterium
5	Rod	+	+	-	+	+	+	+	+	-	-	-	+	Cronobacter condimenti
6	Rod	+	+	-	+	+	+	+	+	-	-	-	+	Cronobacter condimenti
7	Rod	+	+	-	+	+	+	+	+	-	-	-	+	Cronobacter condimenti
8	Cocci	+	+	-	+	-	+	+	-	-	-	+	+	Staphylococcus aureus
9	Rod	-	+	-	+	+	-	-	-	-	-	-	+	Photorhabdus lumiinscences
10	Rod	-	+	-	+	+	-	-	-	-	-	-	+	Photorhabdus lumiinscences
11	Rod	+	-	+	-	+	+	+	+	+	-	-	-	Streptococcus pyogenes
12	Rod	-	+	-	+	+	+	+	+	-	-	-	+	Escherichia coli
13	Rod	-	-	-	+	+	+	+	+	-	-	-	-	Escherichia coli
14	Rod	-	+	-	+	+	+	+	+	-	+	-	+	Cronobacter condimenti
15	Rod	-	+	-	+	+	-	-	-	-	-	-	+	Photorhabdus lumiinscences

16	**Rod**	**-**	**-**	**-**	+	+	+	+	**-**	**-**	**-**	**-**	+	**Haemophilus influenza**
17	**Rod**	**-**	+	**-**	+	+	+	+	+	**-**	+	**-**	+	**Cronobacter condimenti**
18	**Rod**	**-**	+	**-**	+	+	+	+	+	**-**	+	**-**	+	**Cronobacter condimenti**
19	**Cocci**	**-**	**-**	**-**	+	+	+	+	**-**	**-**	**-**	**-**	+	**Haemophilus influenza**
20	**Bacilli**	+	+	**-**	+	+	+	+	+	**-**	+	**-**	+	**Klebsiella aeroginosa**
21	**Rod**	+	**-**	+	**-**	+	+	+	+	+	**-**	**-**	**-**	**Streptococcus pyogenes**
22	**Rod**	**-**	**-**	**-**	+	+	+	+	**-**	**-**	**-**	**-**	+	**Haemophilus influenza**
23	**Rod**	**-**	**-**	+	**-**	+	+	+	+	**-**	**-**	**-**	+	**Escherichia coli**
24	**Cocci**	+	+	**-**	+	**-**	+	+	**-**	**-**	**-**	+	+	**Staphylococcus aureus**

NOTE: G.rxn: Gram reaction, CIT: Citrate; MR: Methyl red; VP:Voges praeuker; GLU: Glucose; LAC: Lactose; SUC: Sucrose; FRUC: Fructose; H2S: Hydrogen Sulphide; GAS: Gas; UREA: Urease; CAT: Catalase.Table 4.1a BIOCHEMICAL

TABLE: 2 Frequency of Isolates from the patients

Bacterial	No of Isolates	%	No of patients	%
Haemophilus influenza	3	12.5	2	10
Staphylococcus aureus	2	8.3	2	10
Escherichia coli	3	12.5	2	10
Streptococcus pyogenes	2	8.3	2	10
Cronobacter condiment	6	25	3	15
Photorhabdus luminscenes	3	12.5	2	10
Klebsiella aeroginosa	2	8.3	3	15
Bacillus tequillensis	1	4.5	1	5
Yersinia molderath	1	4.5	1	5
Bacillus megaterium	1	4.5	2	10