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MICROFLORA ASSOCIATED WITH DIESEL POWERED GENERATORS CONTAMINATED SOIL ARENA OF OBAFEMI AWOLOWO AND ODUDUWA UNIVERSITIES IN ILE –IFE, OSUN STATE, NIGERIA

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ABSTRACT

A total of sixty-five bacterial and fifteen fungal species were isolated from the soil contaminated by diesel oil around power generators in Obafemi Awolowo and Oduduwa Universities both at Ile-Ife, Osun State. Ten soil samples were taken from each university and microflora from them were identified by appropriate biochemical tests and morphological identification. Seven bacteria and fungi genera each were isolated from both top and deep soil. *Bacillus* species were the most predominant bacteria from the samples with 57%, *Staphylococcus* 14%, *Neisseria* 9%, *Micrococcus* 9%, *Corynebacteria* 6%, *Klebsiella* 3%, *Lactobacillus* 2% while *Aspergillus* species were the most predominant of the fungal isolate being 80%, *Penicillium* 16%, *Cunninghamella* 1%, *Humicola* 1%, *Beauveria* 1%, *Gibellula* 1%. It is then concluded that organisms that thrive commonly in all samples of soil analysed particularly *Aspergillus* and *Bacillus spp* can be developed as seed microflora for bioremediation of oil spilled contaminated soil.

Keyword: Isolates, Bioremediation, diesel, contamination, microflora.

1.0 INTRODUCTION

Diesel is a petroleum-based fuel for diesel engines. It is a thick oily fuel that is obtained from the distillation of petroleum. It has an ignition temperature of 540^{o}C and is ignited by the heat of compression. Petroleum diesel or fossil diesel is produced from the fractional distillation of crude oil between 200 °C and 350 °C at atmospheric pressure, resulting in a mixture of carbon chains that typically contain between 8 and 21 carbon atoms per molecule (Collins, 2007). Diesel like all fossil fuels primarily consists of complex mixture of molecules called hydrocarbons. In large concentrations, petroleum products are highly toxic to many organisms, including humans (Alexander, 1994). Diesel oil contains low molecular weight compounds that are usually more toxic than long-chained hydrocarbons, because long-chained ones are less soluble and less bioavailable (Dorn *et al.* 2000). Light oils contain a relatively high proportion of saturated hydrocarbons hence these can be more toxic than heavy oils (Dorn *et al.*, 1998). Petroleum-derived diesel is composed of about 75%; saturated hydrocarbons (primarily paraffins including n, iso, and cycloparaffins), and 25%; aromatic hydrocarbons (including naphthalenes and alkylbenzenes). (ATSDR, 2009) The average chemical formula for common diesel fuel is $C_{12}H_{23}$, ranging approximately from C_{10} H_{20} to C $_{15}H_{28}$. Diesel contains about 38.6 mega joules/litre which gives a higher power. The color of diesel fuels varies from colorless to brown, and the water solubility in 20°C is about 5 mg L-1 and Log Kow 3.3 – 7.06 (ATSDR 1995). Diesel fuels are therefore partly soluble in water and possibly accumulative in tissues. In 2001, UC Riverside installed a 6-megawatt backup power system that is entirely fueled by biodiesel. Backup diesel-fueled generators allow companies to avoid damaging blackouts of critical operations at the expense of high pollution and emission rates.

Soil pollution by petroleum products is a widespread problem. The past decade, bioremediation techniques have been developed and improved to clean up soils polluted with hazardous chemicals (Romantschuk *et. al*., 2000). Excavated petroleum-contaminated soil can be bioremediated by addition of nutrients (biostimulation), addition of microbial inocula (bioaugmentation), aeration and turning, or by a combination of these practices (Alexander, 1994). Pollution often diminishes the total microbial diversity in soil moreover the community composition may be altered by environmental contamination (Lynch *et. al*., 2004). Several bacterial species are capable of oil hydrocarbon degradation because hydrocarbons are naturally produced by plants and microbes (Sylvia *et. al.*, 2005). Biodiversity can be defined as the habitat's animal and plant species and the ecosystems they belong to, and include both species and gene diversity (Ohtonen *et. al*., 1997). In a wide perspective view, this would also include soil microbial biota. Soil microbial biodiversity can be analyzed by cultivating and non-cultivating methods (Vogel. 1996).The dilution plate count method estimates the quantity of microbes, where only viable cells form a colony, but only 0.1 - 10% of microbes are detected by this method (Torsvik *et. al*., 1998). Molecular techniques detect more detailed information about the microbes in an environmental sample (Romantschuk *et. al*., 2000; Saul *et. al*., 2005; Scow & Hicks, 2005).Fungi have much potential for degrading organic compounds, because of their spreading mode of growth and symbiotic association with plants (Sarand *et. al*., 2000; Hosokawa *et. al*., 2009). However, bacteria considered more important colonizers in oil-contaminated soil than fungi (Aislabie *et. al*., 2000). Fungi and bacteria can have a mutualistic relationship, so the presence of fungi can support bacterial growth by the structures of hyphae and mycorrhizae, and some bacteria can improve mycelial growth and so improve the growth conditions of fungi (Sarand *et. al*., 2000). Various pathways are known for the bacterial degradation of different hydrocarbons (Van Hamme *et. al*., 2003; Sylvia *et. al*., 2005).

Pseudomonas bacteria were shown to be important degraders of oil hydrocarbons, especially in cold climates (Aislabie *et. al.,* 2000; Margesin and Schinner 2001; Belousova *et. al*., 2002; Stallwood *et. al.,* 2005; Zhang *et. al.*, 2010). Among other oil degrading bacteria genera are Rhodococcus (Peressutti *et. al.,* 2003; Aislabie *et. al*., 2006) and Sphingomonas (Margesin and Schinner 2001; Aislabie *et. al*., 2006).

The number of studies on composting of petroleum contaminated soil and petroleum-based oil wastes is increasing (e.g. Beaudin *et. al.,* 1996, 1999; Al-Daher *et. al.,* 1998; Kirchmann and Ewnetu, 1998; Milne *et. al*., 1998; Jørgensen *et. al*., 2000; Chaw and Stoklas, 2001; Namkoong *et. al.,* 2002). Elevated temperatures stimulate hydrocarbon degradation (Atlas, 1975), and enhance the contaminant availability by increased solubility and mass transfer (Pignatello and Xing, 1996). Diesel spills usually take place during manufacturing, storage or transportation. Major spills, such as pipeline, tanker or storage tank accidents, create an acute problem of pollution. On the other hand, continuous low-level inputs are rarely noticed, and may pose a serious threat to the environment as contamination accumulates. Therefore, diesel hydrocarbons create a world-wide problem of contaminated water and soil that require decontamination. Contamination along with other physical and chemical changes in soil can cause adaptive changes in the composition of the bacterial community (Saul *et. al*., 2005; Grant *et. al*., 2007). Competition within and between species influences the bacterial community composition and hence degradation of contaminants. Soil pore size influences degradation by providing surface to colonize and because of predation that reduces bacterial biomass especially in pores that are larger than 2µm (Johnsen *et. al.,* 2005). Therefore in soils where the pore size is large, there are fewer microbes available for degradation than in soils with small pore size.

1.1 Microorganisms Implicated In the Biodegradation of Oil in the Soil

Some microorganisms are equipped with metabolic machinery to use petroleum hydrocarbon as carbon and energy source. Hydrocarbons in their natural environment are degraded by bacteria and fungi. The fraction of the total heterotrophic communities represented by the hydrocarbon utilizing bacteria and fungi is highly available with reported frequency ranging from 6%-82% for soil fungi, 0.13%-50% for soil bacteria and 0.33%-100% for marine bacteria. Most important hydrocarbon degrading bacteria belong to the following genera; *Alcaligenes, Achromobacter, Bacillus, Arthrobacter, Flavobacterium, Pseudomonas, Corynebacterium, Beinjerinchia*. The hydrocarbon utilizing fungi includes the following; A*ureobasidium, candida, Sporolobomyces, Rhodotorula, Trichoderma, Mortiella.* Some Hydrocarbon Degrading Bacteria Isolated From Soil;*Pseudomonas, Bacillus, and Acinetobacter* (Anupama and Padma, 2002) *Flavobacterium, Acinetobacter and Pseudomonas* (Mandri and Lin. 2006) *Yokenella, Alcaligenes, Roseomonas, Stenotrophomonas, Acinetobacter, Flavobacterium,Corynebacterium, Streptococcus, Providencia, Sphingobacterium, Capnocytophaga, Moraxella and Bacillus*(Rusanky *et. al.,* 1987; Antai, 1993; Bhattacharya *et. al.,* 2002) *Pseudomonas, Streptococcus, Escherichia coli, Staphylococcus, Klebsiella, Bacillus, Mycobacterium, Enterobacteraerogenes, Salmonella, and Micrococcus (*Makut and Ishaya, 2010).

2.0 MATERIALS AND METHODS

2.1 Sampling Sites

The soil samples for this study were collected from two different locations of diesel powered generators Obafemi Awolowo University ile-ife set up in 1960 and Oduduwa University Ipetumodu set up in 2010. Soil samples were taken from nearby of the generators and disposal areas contaminated with diesel oil waste. Five soil samples each from surface (0-5 cm depth) and deep soil (15-20cm depth) were collected from each power house at 5m distance apart. The samples were taken consecutively after tilling with a sterile scoop and transferred into sterile polythene bags for microbiological determination. 1gram of each soil sample was aseptically transferred to 9ml of sterile water for serial dilution process and then cultured on PDA and NA for fungal and bacterial isolates respectively. Sub-culturing was done until pure isolates were obtained which were later stored on slants in the refrigerator at 4^{o}C for identification.

2.2 Isolation and Identification of Bacterial and Fungal Isolates

The identification of hydrocarbon utilizing bacterial was based on biochemical characterization such as sugar and alcohol sugars fermentation tests, citrate, catalase, indole, methylred, voges-prauskauer, starch hydrolyses, oxidase etc. The identification of hydrocarbon utilizing fungal isolates was based on colonial appearances, wet mount preparation and the use of different staining technique such as Methylene cotton-blue, Indian Ink/Nigrosin Ink (Chukwura *et al*., 2004).

2.3 Staining Reaction

The following reactions were carried out to know the gram reaction and spore formations of the isolates.

2.4 Gram's Staining Reaction

Smears of 24hour old isolates were on slides and heat fixed. Crystal violet was first applied and allowed to remain for 1 minute and washed off with water. Grams iodine was added. This stayed for another 1 minute and was later washed off. The smear was decolorized with alcohol, washed with water and counter stained with 1% Safranin. Smear was them blot dried with clean filter paper and observed under (X100) oil immersion lens of the microscope.

2.5 Spore Staining

This staining procedure was carried out to confirm the presence or absence of spores produced by the isolates. Spores production was induced by adding 0.5ml of 0.4% solution of Manganese sulphate ($MnSO_4$ $4H_20$) per litre of medium .Smear from the medium was heat fixed and flooded with Malachite green. The slides were heated until the stain started to boil. The stain was then washed off and the smear was counter-stained with safranin. Bacterial spores stained

green while vegetative cells stained red on examination under the microscope. The shapes and positions of endospore were observed.

2.6 Motility Test

Motility of the isolates were determined using cavity-slide method. A drop of 24hours old bacteria suspension was placed on a cover slip and inverted over a depression of a cavity slide, so that the drop of bacteria suspension formed a hanging drop on the cover slip. The drop was observed for activity motile cells under (X100) oil immersion objective of a microscope.

2.7 Biochemical Tests

The isolates were made to undergo a number of biochemical tests as follows.

2.7.1 Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdown of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of 3% H_20_2 was added to a 24 hour old culture of isolates on slide. Evolution of a gas white froth indicated a catalase positive reaction while the absence of the effervescence or white froth showed negative reaction.

2.7.2 Voges-Proskauer Test

This is a test carried out to know if the isolates can produce acetymethylcarbinol from glucose. The medium used is glucose phosphate broth. The sterilized medium was distributed into test tubes in 5ml volumes and inoculated with the isolates, an uninoculated tube served as control.

2.7.3 Methyl-red Test

This test was used to determine whether the production of acid glucose has lowered and help pH at about 4.2 or below. The medium used is glucose-phosphate broth. This was distributed into screw caps and was sterilized at 121^0C for 15 minutes. Isolated were grown in the medium for two days after which methyl-red test reagent was added. Uninoculated tubes served as control. Development of yellow colour was recorded as negative result.

2.7.4 Starch Hydrolysis

This test was carried out to determine the production of amylase by the isolates. Starch agar plates were made by dissolving and melting starch to gelatinization in a known volume of distilled water and adding to a melted nutrient agar to give a final concentration of 1% starch. The medium was autoclaved and poured into sterile plates before solidifying. Single streak of isolates was done on plate and incubated at 37^0C for 48 hours, after which plates were flooded with Gram's iodine. Unhydrolysed starch formed a blue colour with the iodine. Clear zones around the area of growth indicated starch hydrolysis and are the results of B-amylase activity while reddish brown zones around the area of growth indicated partial hydrolysis of starch i.e. result of e-amylase.

2.7.5 Oxidase Test

A few drops of the oxidase reagent (1% aqueous tetramethyl-p-phenylene diamine hydrochloride) was added onto an isolated colony from 24 hours old pure culture. A purple coloration produced within five-ten second on clean filter paper indicated oxidase positive cultures. A delayed reaction was recorded as negative.

2.7.6 Indole Test

Indole is a nitrogen-containing compound formed from the degradation of the amino acid tryptophan by various bacteria. Tryptone broth was prepared and distributed into screw caps tubes and sterilized at 121^0C for 15mins. After cooling, the tubes were inoculated and incubated at 350C for 48 hours. After which 3ml of Kovac's reagent was added to 6ml of the culture fluid. The mixture was mixed by rotating the tube between the palms. A reddening of the alcohol layer within five minutes indicated indole production in the tubes. Control tube was set up and no reddening layer was observed.

2.7.7 Nitrate Reduction

Nitrate is reduced by some microorganisms to nitrite, ammonia on free nitrogen. The medium contained 0.1% KNO_3 in peptone water. This was distributed into test tubes fitted with inverted durham tubes. The tubes were autoclaved cooled and inoculated with the isolates. Uninoculated tubes served as controls. The tubes were incubated at 35^0C for 4 days. The presence of nitrite was determined by the addition into each tube of 0.5ml of 1% sulphanilic acid in 5N acetic acid. This was followed by 0.5ml of 0.6% dimethyl-naphthylamine in 5N acetic acid. The development of a red colour showed the presence of nitrite. Gas production was indicated by its accumulation in the durham's tubes.

2.7.8 Sugar Fermentation Test

The test is used to determine the ability of the bacteria and yeasts to utilize different sugars. This is useful in distinguishing the different strains of organisms. 10% solution of each sugar was separately prepared and 1m1 of each sugar was added to 9mls tubes of phenol red broth base which had been separately sterilized at 121^0C for 10 minutes while the phenol broth was sterilized at 121^0C for 15mins in screw capped tubes. Each tube was fitted with an inverted Durham tube. The tubes were inoculated with test organisms (bacteria and yeasts cultured) and incubated for 3 days at

35^0C. Uuninoculated tubes served as controls. Acid production was indicated by a change of colour medium from red to yellow. Production of gas was collected in the inverted Durham's tubes.

2.7.9 Urease Test

Urease Christenson's urea slope is used for this experiment. This was done by preparing glucose phosphate agar with o.4% phenol red and 40% urea added. Where urea is produced, the ammonia liberated makes the indicator to turn re. In this experiment liquid modification of Christenson's medium was sed. It assayed urea-splitting organisms and urea hydrolysis by other microorganisms. Urea broth base with the following compositions: Peptone: 1g/ltr., Dextrose: 1g/ltr., Sodium dihydrogen Phosphate: 0.8g/ltr., Disodium phosphate. 1.2g/ltr. Sodium chloride 5g/ltr., Phenol red 0.004g/ltr. Was mixed and prepared. 49% urea was prepared, sterilized and 100ml of this was added to 1000ml of sterilized urea broth base. This was mixed thoroughly and dispensed aseptically into sterile MacCartney bottles. The bottles were then inoculated from peptone water culture incubated for 48 hours earlier. Positive result gave a red colouration of the medium. This indicated urea-splitting or urea hydrolysis, control set up under the same condition didn't turn red, that is, the colour remained unchanged.

2.7.10 MR/VP Test

MR indicates methyl red test while VP indicates Voges-proskaur test. The VP is test for the production of acetylmethylcarbinol or acetoin and this maybe tested for in the same culture as used for MR tests. The coli and aerogenes bacteria are similar and MR/VP tests helps to distinguish them from each other. Many of the coliform organisms ferment dextrose rapidly causing a fall in pH of the medium. The acidic reaction of the medium is checked using methyl red indicator. Acetoin is produced by the condensation of two molecules of pyruvic acid which is a product of the metabolism of dextrose, a characteristic possessed by the aerogenes groups.

Treat for M

5 drops of methyl red solution was added and examined for colors, red indicates positive (i.e acid produced) and yellow color indicates negative result and was recorded.

Treat for V.

5 drops of KOH was added and another 5 drops of barrits solution was added. The development of pink color within 30 minutes indicated positive result.

2.7.11 Sodium Chloride test

This test determines whether the microbe can grow in a medium containing 6.5% sodium chloride (NaCl), also known as table salt. The medium used was 6.5% NaCl broth. It is a nutrient broth containing 6.5% sodium chloride, or table salt.An inoculum from a pure culture was transferred aseptically to different sterile test tubes of 6.5% NaCl broth the inoculated tube is incubated at 35-37 C for 24 hours. A positive test is indicated by the presence of turbidity.

2.7.12 Bacteria Spore Stain

Some groups of bacteria usually members of bacillus and clostridium produce endospore which was relatively resistant to the common stains however the spore walls can be made permeable to these stains by heating the smear preparation. After preparing the smear, heat fixed the organism to the slide, I added malachite green solution and steamed for 10 and washed off carefully with slightly running tap water and I counterstain with safranin solution for 15 seconds after which I washed off with tap water and examined under the microscope using the oil immersion objective and I put a drop of lmmersion oil. To observe, the spore with green indicates positive while spore with red indicates negative.

3.0 RESULTS AND DISCUSSIONS

A total of sixty five bacterial isolates (forty gram positive rods, thirteen gram positive cocci, two gram negative rods and ten gram negative cocci) and fifteen fungal isolates were obtained from the soil samples analysed. The microbial load obtained from the analysis of the soil samples obtained from diesel powered generator site ranged from $3.8x10^{-5}$ to $9.5x10^{-7}$ cfu/g. This indicates a profound presence of bacteria and fungi population in soil contaminated with diesel. According to Das and Mukherjee (2006), contaminated site often harbour a vast array of microbial flora that is capable of utilizing diesel as a carbon and energy source. Below are tables showing results for various morphological characteristics and biochemical tests performed for the isolation and identification of individual microbes. B1 to B10 are isolates from OUI while B11 to B19 are isolates from OAU. Capital letters are used to represent isolates from top soil while small letters are used for isolates from deep soil.

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Insert Table 3:1 Here

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The fungi that were isolated from both power house soil are as follows; *Aspergillus spp, Penicillium spp, Fusarium spp, Humicola spp, Gibelllula spp, Cunninghamella spp* and *Beauveria spp* and all the fungi are found on soil

contaminated with hydrocarbons and are known to degrade hydrocarbons. Previous study on isolation of fungi showed that; *Aspergillus* (Bartha and Atlas, 1977; Obire *et al*., 2008) *Cunninghamella* (Bartha and Atlas, 1977), *Fusarium* (Llanos and Kjoller, 1976; Obire *et al*., 2008a) *Penicillium* (Llanos and Kjoller, 1976; Bartha and Atlas, 1977; Obire et al., 2008) are in accordance with present study.

A study by (Wemedo *et al*., 2002) also recorded that genera of fungi such as Penicillium and Aspergillus spp are associated with kerosene-polluted soil. Oboh *et al*., (2006) reported all of these isolates were able to grow on crude petroleum as the sole source of carbon and energy when screened for hydrocarbon utilization. Aspergillus and Penicillium spp have frequently been isolated from marine and terrestrial environments. The advantages associated with fungal bioremediation lay primarily in the versatility of the technology and its cost efficiency compared to other remediation technologies (such as incineration, thermal desorption, extraction) (Aust, 1990) More so, their utilization is a gentle non-aggressive approach. The application of bioremediation capabilities of indigenous organisms to clean up pollutants is viable and has economic values (Bijofp, 2003). Also, Okerentugba and Ezeronye (2003) demonstrated that *Penicillium* spp. and *Aspergillus* spp were capable of degrading hydrocarbons especially when single cultures were used. In petroleum-producing regions of Nigeria, Obire (1988) found several species oil-degrading aquatic fungi in the genera of *Aspergillus niger, Aspergillus terreus,Fusarium sp., Penicillium spp* which is in agreement with present work.

Batelle (2000) showed that fungi were better degraders than traditional bioremediation techniques including bacteria, unlike other groups of microorganisms, filamentous fungi do not exhibit preferential degradation for particular chain lengths of alkanes. Fungal mycelia penetrate oil and increase the surface area available for degradation by other microbes. Fungi are notably aerobic and can also grow under environmentally stressed conditions such as low pH and poor nutrient status, where bacterial growth might be limited. Furthermore, fungi are easy to transport, genetically engineer, and produce in large quantities. (Obire and Ramesh. 2009). The ubiquitous distribution of bacteria and fungi with their ready isolation from oil-contaminated environments indicate that they play an important role in the degradation of oil spilled in the environment.

Bacteria and fungi have evolved the ability to degrade petroleum hydrocarbons, the objectives of this study were therefore, to isolate and identity some of the indigenous bacterial and fungal flora of oil contaminated soils and evaluates the biodegradation efficiencies of the potent isolates

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ANNEXURE

Table 3.1: Biochemical Characteristics for Gram Positive Rods

S/N	Isolates	Gram stain	Catalase	Starch	Spore	Glucose	Mannitol	Voge-pros	Nitrate	Citrate	NaCl	Presumptive Identity
1	B1	+	+	+	-	A	Ng	+	+	-	+	*Corynebacterium kutsceri*
2	B3	+	+	-	-	A	Ng	+	-	-	+	*Corynebacterium xerosis*
3	b4	+	++	-	+	AG	Ng	+	+	-	+	*Bacillus pasteurii*
4	c4	+	+	+	+	A	A	-	-	+	+	*Bacillus circulan*
5	D4	+	+	+	+	A	Ng	+	+	-	+	*Bacillus alvei*
6	d4	+	+	+	+	AG	AG	-	+	+	+	*Bacillus mecerans*
7	d5	+	+	-	+	AG	AG	-	-	-	-	*Bacillus sphaericus*
8	e1	+	+	-	-	A	A	-	+	-	+	*Bacillus insolitus*
9	e2	+	-	+	+	A	Ng	+	+	+	+	*Bacillus licheniformis*
10	e3	+	-	+	-	A	A	-	-	+	-	*Lactobacillus casei*
11	F2	+	+	+	-	AG	AG	-	-	+	-	*Bacillus megaterium*
12	F3	+	+	-	+	AG	AG	+	+	+	+	*Bacillus pasteurii*
13	f3	+	+	-	-	A	Ng	-	+	-	+	*Bacillus insolitus*
14	f5	+	+	-	-	A	Ng	-	+	-	+	*Corynebacterium xerosis*
15	G2	+	+	-	+	A	A	+	-	-	+	*Bacillus sphaericus*
16	G3	+	+	+	+	A	Ng	-	+	-	+	*Bacillus circulans*
17	H2	+	+	+	+	AG	Ng	+	+	-	+	*Bacillus alvei*
18	H4	+	+	+	+	A	AG	-	+	+	+	*Bacillus macerans*
19	I3	+	+	-	-	A	AG	-	+	+	+	*Bacillus marinus*

Gr=Gram stain; Cat=catalase test(positive+/ negative-); sta=starch hydrolysis test(positive+) /Negative-); sp=spore(positive+/ negative-); Man= mannitol test(AG=acid and gas production or A=acid production and Ng= no growth); Glu=glucose test(AG= acid and gas production or A=acid production and Ng=No growth); Nit=nitrate reduction test(positive +/negative-); VP=voges proskaur test; cit=citrate test; Na=6.5 Nacl test.

Table 3.2: Biochemical characteristics for Gram positive Cocci.

S/N	ORGANISM	GRAM	CATALASE	GLUCOSE	MANNITOL	Presumptive Identity
1	B2	+ Cocci	+++	A + G	A + G	*Staphylococcus aureus*
2	B5	+ Cocci	+++	A	NG	*Micrococcus varians*
3	b1	+ Cocci	+	A	NG	*Micrococcus varians*
4	b3	+ Cocci	+	A	A	*Staphylococcus aureus*
5	d3	+ Cocci	+++	A + G	NG	*Micrococcus varians*
6	F1	+ Cocci	++	A + G	NG	*Staphylococcus aureus*
7	f1	+ Cocci	++	A	NG	*Micrococcus varians*
8	f2	+ Cocci	++	A	NG	*Micrococcus varians*
9	f4	+ Cocci	+	A	A + G	*Staphylococcus aureus*
10	G1	+ Cocci	+	A + G	A + G	*Staphylococcus aureus*
11	g2	+ Cocci	+++	NG		*Micrococcus luteus*
12	g3	+ Cocci	+++	A + G	A + G	*Staphylococcus aureus*
13	j3	+ Cocci	+	A + G	A	*Staphylococcus aureus*

Table 3.3: Biochemical Characteristics of Gram Negative Rods

S/N	Isolate	Gram stain	Oxidase	Catalase	Urease	Mannitol	Glucose	Methyl Red	Voge Proskaur	Indole	motility	H_2S	Lactose	Citrate	Identity (Presumptive)
1	c3	-	-	+	-	-	+	+	-	-	-	-	+	-	*Klebsiella pneumonia*
2	J2	-	-	+	+	-	+	-	+	+	-	-	+	+	*Klebsiella oxytoca*

Gr=Gram stain; Cat=catalase test(positive+/ negative-); Oxd=Oxidase test; Man= mannitol fermentation test(AG=acid and gas production or A=acid production and Ng= no growth); Glu=glucose fermentation test; MR= Methyl red test; VP=voges proskaur test; cit=citrate test; Ind= indole test; Lac= lactose fermentation test, ur=urease test; Mol= Motility test; H_2S = hydrogen sulphide production test.

Table 3.4 Biochemical Characteristics of Gram Negative Cocci.

S/N	Organisms (Presumptiv)	Gram	Catalas	Glu	Man
1	Neisseria	-	++	A	A
2	Neisseria	-	++	A	A
3	Neisseria	-	+	A	A
4	Neisseria	-	++	A	A
5	Neisseria	-	++	A G	A
6	Neisseria	-	++	A	A G
7	Neisseria	-	+	A G	A G
8	Neisseria	-	++	A G	A
9	Neisseria	-	+++	A G	A
10	Neisseria	-	++	A	A G

Gr=Gram stain; Cat=catalase test(positive+/ negative-); Man= mannitol fermentation test(AG=acid and gas production or A=acid production and Ng= no growth); Glu=glucose fermentation test(AG= acid and gas production or A=acid production and Ng=No growth).