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# Heamolytic activities, deoxyribonuclease production and in-vitro flouroquinolones susceptibility profile of aerobic gram positive cocci associated with acne vulgaris

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ABSTRACT

Bacteriological investigations were carried out on forty–six (46) swabbed samples from the pustular and nodulocystic skin lesions of undergraduate students with acne vulgaris using standard bacteriological methods. The fluoroquinolones susceptibility profiles, heamolytic activities, deoxyribonuclease production were determined using Kirby –Bauer disc diffusion methods, Columbia Blood Agar (CBA) and Deoxyribonuclease (DNase) agar, respectively. The results showed that all the 46 clinical samples showed positive growth, with 24 (52.2%) having single bacterium isolated. Co-infection with two bacterial species was seen in 24 (28.3%), while polybacterial growth was present in 9 (19.6%) of the samples. Staphylococcus aureus was the most prevalent aerobic Gram positive cocci associated with acne vulgaris with 32 (42.1%) occurrences, followed by Staphylococcus epidermidis 26 (34.2%) and Micrococcus spp 18 (23.9%). Sixteen of the isolates produced α – haemolysin, 25 (32.9%) produced β – haemolysin and 35 (46.1%) produced no haemolysin (γ-haemolysis). Of the 16 α – haemolysin producers, Staphylococcus aureus produced the highest 9 (56.3%), followed by Staphylococcus epidermidis 5 (31.3%) and Micrococcus spp 2(12.5%). Of the seventy – six aerobic Gram positive cocci isolated only 33 (43.4) were DNase producers, while 43 (56.6) were non-DNase producers. Staphylococcus aureus were highly sensitive to Moxifloxacin, while 26 (81.3%) were sensitive to Lomefloxacin and Gatifloxacin. Staphylococcus epidermidis and

Micrococcus spp were highly sensitive to Gatifloxacin, Moxifloxacin, Lomefloxacin and Levofloxacin. The results also showed that 17 (65.4%) and 13 (50.0%) of Staphylococcus epidermidis were sensitive to Pefloxacin and Nalidixic, respectively. Consequently, this study has shown that fluoroquinolones could be drugs of choice to administer to patients with acne vulgaris.



## 1. Introduction

Acne vulgaris (pimples) is a chronic disorder of the hair follicles and sebaceous glands or a disease of features known as pilosebaceous units which are numerous on the face, upper back and chest (Webster, 1998; Asima *et al*., 2011). Acne is characterized by areas of skin with multiple non-inflammatory follicular papules and by inflammatory papules, pustules and nodules in its more severe forms (Bettoli *et al.,* 2006). Acne is the most common skin disorder in the world with prevalence about 70-87% (Dreno and Poli, 2003). Although, it is not a serious threat to general health but it is one of the most socially and psychologically distressing skin conditions, especially for adolescents, resulting in diminished self esteem, social withdrawal due to embarrassment and depression (Hassanzadeh *et al.,* 2008; Bowe and Logan, 2011). Teenagers are more susceptible to acne vulgaris development for their diets consist primarily of processed foods that are deficient in the basic micro nutrients and out of balance with regard to the macro nutrients of fat, protein and carbohydrate, required to create both hormones and healthy skin, thus making their skins to be nutritionally deprived and highly prone to developing severe acne (Pochi, 1990; Ferdowsan and Levin, 2010).

Studies have shown that both aerobic and anaerobic bacteria could be associated with acne vulgaris (Ross *et al*., 2001; Farrar, 2007). Healthy skin pores are colonized by *Propionibacterium acne,* a relatively slow growing aero-tolerant anaerobic Gram positive bacterium, while non-pore resistant *Staphylococcus epidermidis*, *Staphylococcus aureus,* and *Micrococcus* spp sometimes are sometimes found from the culture of the follicular material (Silverberge and Weinberg, 2001; Hiramatsu, 2003; Farrar, 2007). These organisms increase the inflammatory reaction that may be initiated by increase in sebum production, free fatty acids from sebum itself and abnormal keratinization of the sebaceous canal. Some pathogens such as *Staphylococcus aureus*, possess a wide array of virulence factors e. g DNases and haemolysins, which cause damage to host tissues (Prescott *et al*., 2008). The haemolytic activity of the bacterial isolates could be either partial (alpha haemolysis) or complete (beta haemolysis) breaking down of red blood cells (Ray *et al.,* 2004). Alpha haemolysis is caused by hydrogen peroxide produced by the bacterium, oxidizing hemoglobin to green methamoglobin (Ray *et al.,* 2004; Prescott *et al*., 2011).

Fluoroquinolones are a family of potent synthetic broad spectrum antibiotics or antimicrobial agents, usually administered orally or sometimes intravenously for treatment of selected community acquired, nosocomial or serious infections (Nelson *et al.,* 2007). The targets of flouroquinolones activity are the bacterial DNA gyrase and topoisomerase IV enzymes essential for DNA replication and transcription (Nelson *et al.,* 2007; Akinjogunla and Eghafona, 2011). Due to the development of resistance in microorganisms causing acne to common antibiotics and the differences in species and strains of the microorganisms in different regions, a research in the method of therapy seem indispensable (Ross *et al.,* 2001). Consequently, this study was carried out to ascertain the Gram positive aerobic bacteria associated with acne, the susceptibility of the bacteria to flouroquinolones, their heamolytic and deoxyribonuclease producing capabilities.

## 2. Materials and methods

Forty–six (46) swabbed samples from the pustular and nodulocystic skin lesions of undergraduate students (Age ranged 16-30 years) with acne vulgaris were collected from July, 2011 to September, 2011 under aseptic conditions and inoculated into broth cultures for 4-6hrs and later inoculated onto plates of Mannitol Salt Agar (MSA) and Blood Agar (BA). The plates were incubated aerobically for 24 hrs at 37°C. After overnight incubation, the Mannitol Salt Agar plates were examined for fermentation of mannitol indicated by colour change of the medium around each colony from red to yellow (For *Staphylococcus* spp). The colonies on the both Mannitol Salt

Agar (MSA) and Blood Agar (BA) plates were sub-cultured onto nutrient agar plates and further speciated by conventional laboratory techniques including Gram staining; catalase test, coagulase test, urease production, indole production, gelatin hydrolysis, citrate utilization and Vogues-Proskauer test, coagulase test and biochemical tests (mannitol, sucrose, maltose, lactose, glucose and galactose).

**2.1. Sterilization of glass wares**

All the glass wares used were thoroughly washed with detergent and rinsed with clean water before usage. The glasswares such as test tubes, conical flasks, Petri dishes, beakers, pipettes, Durham's tube and McCartney bottles were sterilized in the hot air oven at $180^0$C for 1½ hour. The wire loop was flamed to redness before and after usage.

**2.2. Haemolytic activity of the bacterial isolates**

The haemolytic activity of the bacteria (*Staphylococcus aureus, Staphylococcus epidermidis* and *Micrococcus* spp) was identified by the presence of clear (β – haemolysis) or green colouration (α– haemolysis) halos around the colonies on Columbia blood agar (Oxoid , UK) supplemented with 5% sheep blood. The bacterial suspensions were streaked onto Columbia blood agar plates and incubated for 24 hours at $37^0$C. Observations of the haemolytic zone around colonies after incubation were made and the type of haemolysin was recorded as α, β and no haemolysis as γ.

**2.3. Detection of deoxyribonuclease (DNASE) producing isolates**

DNase agar (Oxoid, UK) was used for detection of microbial deoxyribonuclease enzymes. Spot inoculation were done onto to the surface of the DNase agar medium and incubated at $37^o$C for 48hrs. After incubation, the growth on the surface of the agar was flooded with 1N hydrochloric acid. Polymerized DNA precipitated in the presence of 1N HCl and made the medium opaque. The bacteria that produced DNase enzymes, in sufficient quantity to hydrolyse DNA with clear zones around the colonies were evaluated as positive.

**2.4. Antibiotics susceptibility test**

*In-vitro* susceptibility profile of *Staphylococcus aureus, Staphylococcus epidermidis* and *Micrococcus* spp. isolated to four different generations of fluoroquinolones (antibiotic) was determined using Kirby Bauer disc diffusion technique (Bauer *et al.,* 1996). Thirty- six (36) grams of Muller Hinton Agar was dissolved in 1 litre of distilled water and autoclaved at $121^0$C for 15minutes. The agar was poured aseptically into sterile Petri dishes and allowed to solidify. Zero point one (0.1) ml of each bacterial isolates prepared directly from an overnight agar plate and adjusted to 0.5 McFarland Standard was inoculated using sterile pipette onto Petri dishes containing Muller Hinton Agar and was allowed to stand for 30minutes to enable the inoculated organism to pre-diffuse. The fluoroquinolones discs (5ug) : $I^{st}$ generation (Nalidixic); $2^{nd}$ generation (Lomefloxacin, Pefloxacin , Norfloxacin, Ciprofloxacin); $3^{rd}$ generation (Sparfloxacin , Levofloxacin) and fourth generation (Gatifloxacin , Moxifloxacin ) were aseptically placed on the surfaces of the sensitivity agar plates using a sterile forceps and gently pressed to ensure even contact. The plates were incubated at $37^0$C for 24 hours and the zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimeters (mm) using a ruler. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative manual. Percentage resistance was calculated using the formula PR = a / b X 100 "PR" is % resistance, 'a' is the number of resistance isolates and 'b' is the number of isolates tested with the antibiotics. The percentage (%) sensitivity was calculated using the formula PS = c/d X 100 where "PS" is % sensitivity, 'c' is the number of sensitive isolates and 'd' is the number of isolates tested with the antibiotics (Akinjogunla and Enabulele, 2010).

**3. Results**

On the basis of cultural, morphological and biochemical characteristics, three aerobic Gram positive cocci (*Staphylococcus aureus, Staphylococcus epidermidis* and *Micrococcus* spp.) were obtained from subjects with pustular and nodulocystic skin lesions (Table 1). The results showed that *Staphylococcus aureus* was the most prevalent aerobic Gram positive cocci associated with acne vulgaris with 32 (42.1%), occurrences, followed by

**Table 1**
Morphological and biochemical characteristics of aerobic gram positive cocci associated with acne vulgaris.

| **Morphology / Biochemical Tests** | ***taphylococcus aureus*** | ***Staphylococcus epidermidis*** | ***Micrococcus* spp** |
|---|---|---|---|
| Shape | Coccoid in cluster | Coccoid in cluster | Coccoid in tetrad |
| Gram Staining | +ve | +ve | +ve |
| Mannitol | +ve | -ve | +ve |
| Sucrose | +ve | +ve | +ve |
| Maltose | +ve | +ve | +ve |
| Lactose | +ve | +ve | -ve |
| Galactose | +ve | +ve | +ve |
| Glucose | +ve | +ve | +ve |
| Catalase | +ve | +ve | +ve |
| Coagulase | +ve | -ve | -ve |
| Indole | -ve | -ve | -ve |
| Citrate | +ve | +ve | +ve |
| Methyl red | +ve | +ve | -ve |
| Vogues-Proskauer | -ve | -ve | -ve |
| Gelatin hydrolysis | +ve | +ve | +ve |

+ve = positive
-ve = negative

**Table 2**
Prevalence and heamolytic activities of aerobic gram positive cocci associated with acne vulgaris.

| **Bacterial Isolates** | **No (%) of Occurrence** | **Types of Heamolysin** | | |
|---|---|---|---|---|
| | | **α** | **β** | **γ** |
| | | **No (%)** | **No (%)** | **No (%)** |
| *Staphylococcus aureus* | 32 (42.1) | 9 (28.1) | 19 (59.4) | 4 (12.5) |
| *Staphylococcus epidermidis* | 26 (34.2) | 5 (19.2) | 4 (15.4) | 17 (65.4) |
| *Micrococcus* sp | 18 (23.9) | 2 (11.1) | 2 (11.1) | 14 (77.8) |
| Total | 76 (100) | 16 (21.1) | 25 (32.9) | 35 (46.1) |

Values in parenthesis are percentages

**Table 3**
Prevalence deoxyribonuclease producing aerobic gram positive cocci associated with acne vulgaris.

| Bacterial Isolates | No (%) of Occurrence | Number / Percentages of DNase Producers | Non DNase Producers |
|---|---|---|---|
| *S. aureus* | 32 (42.1) | 26 (81.2) | 6 (18.7) |
| *S. epidermidis* | 26 (34.2) | 5 (19.2) | 21 (80.8) |
| *Micrococcus* spp | 18 (23.9) | 2 (11.1) | 16 (88.9) |
| Total | 76 (100) | 33 (43.4) | 43 (56.6) |

*Staphylococcus epidermidis* 26 (34.2%) and *Micrococcus* spp 18 (23.9%) (Table 2). The occurrence, percentage and type of haemolysins produced by the aerobic Gram positive cocci associated with acne vulgaris using Columbia blood agar plates are shown in Table 2.

The results showed that 16 (21.1%) of the isolates produced α – haemolysin, 25 (32.9%) produced β – haemolysin and 35 (46.1%) produced no haemolysin (γ-haemolysis). Of the 16 α – haemolysin producers, *Staphylococcus aureus* produced the highest 9 (56.3%), followed by *Staphylococcus epidermidis* 5 (31.3%), and *Micrococcus* spp 2(12.5%). Among the β- haemolysin producers, *Staphylococcus aureus* had the highest 19 (76.0%), followed by *Staphylococcus epidermidis* 4 (16.0%), while *Micrococcus* spp had 2 (8.0%). Table 2 also shows that 4 (12.5%), 17 (65.4%) and 14 (77.8%) of *Staphylococcus aureus, Staphylococcus epidermidis* and *Micrococcus* spp produced no haemolysin (γ- heamolysis), respectively. The prevalence of deoxyribonuclease producing aerobic Gram positive cocci associated with acne vulgaris is shown in Table 3. Of the seventy – six aerobic Gram positive cocci isolated only 33 (43.4%) were DNase producers, while 43 (56.6%) were non- DNase producers (Table 3).

The clinical source of isolation (face, back and chest), age, sex and bacteria isolated from the subjects with acne vulgaris are shown in Table 4. All the 46 clinical samples showed positive growth, with 24 (52.2%) having single bacterium isolated. Co-infection with two bacterial species was seen in 24 (28.3%), while polybacterial growth was present in 9 (19.6%) of the samples.

The results of the *in vitro* susceptibility profile of the aerobic Gram positive bacteria associated with acne vulgaris are shown in Figs i-iii. The results showed that between 56.3 to 62.5% *Staphylococcus aureus* were sensitive to Nalidixic and Ciprofloxacin (Fig. 1) *Staphylococcus aureus* was highly sensitive to Moxifloxacin, while 26 (81.3%) were sensitive to Lomefloxacin, Levofloxacin and Gatifloxacin (Fig. 1). The sensitivity of the *Staphylococcus epidermidis* isolated from subjects with acne vulgaris in decreasing order are as follows: 22 (84.6%) were sensitive to Gatifloxacin, 21 (80.8%) were sensitive to Moxifloxacin and Levofloxacin; 20 (77.0%) were sensitive to Sparfloxacin; 19 (73.1%) were sensitive to Lomefloxacin, Ciprofloxacin and Norfloxacin; 17 (65.4%) were sensitive to Pefloxacin, while 13 (50.0%) were sensitive to Nalidixic (Fig. 2).*Micrococcus* spp was highly sensitive to Gatifloxacin, Moxifloxacin, Lomefloxacin and Levofloxacin while moderate sensitivity of *Micrococcus* spp to Norfloxacin, Sparfloxacin, Ciprofloxacin and Pefloxacin was observed (Fig. 3).

## 4. Discussion

Skin is perhaps the most vulnerable part of the body and day to day exposure of skin leads to number of problems such as acne. Acne, a common skin disease with potential complications has many psychiatric and psychological implications than most other dermatological conditions. In this study, *S. aureus, S. epidermidis* and *Micrococcus* spp were obtained from the subjects with pustular and nodulocystic skin lesions and the isolation of these aerobic Gram positive cocci from acne is in agreement with Hassanzadeh *et al*. (2008), who reported *S. aureus* , *S. epidermidis*, and *Micrococcus* spp as the commonest aerobic organisms associated with acne vulgaris. The occurrence of *S. aureus* from pores of subjects with acne in this study also agrees with Asima *et al*. (2011). Bek-Thomson (2008) indicated that *S. epidermidis* was universally found inside affected acne vulgaris pores and this study confirmed it.

Acne vulgaris is a polymorphic disease that occurs on the face, back and chest (Shweta and Swarnlata, 2011). The isolation of aerobic Gram positive cocci from the face, back and chest of acne subjects is in conformity with Shweta and Swarnlata (2011).

**Table 4**
Clinical source of isolation, age/sex and bacteria isolated from subjects with acne vulgaris.

| Sample Codes | Sex/Age | Sources | Bacteria Isolated |
|---|---|---|---|
| AV-01 | F/18 | Face | a, b, c |
| AV-02 | F/ 16 | Face | b |
| AV-03 | M/22 | Face | a, b |
| AV-04 | F/ 22 | Back | a, c |
| AV-05 | F/23 | Back | a, b, c |
| AV-06 | M/24 | Chest | a |
| AV-07 | M/21 | Back | b |
| AV-08 | M/19 | Face | a |
| AV-09 | M/25 | Back | a, b, c |
| AV-10 | F/ 26 | Face | b |
| AV-11 | F/ 20 | Face | a |
| AV-12 | M/23 | Face | a |
| AV-13 | F/24 | Face | a , c |
| AV-14 | F/21 | Chest | a |
| AV-15 | F/25 | Face | a, b, c |
| AV-16 | F/27 | Chest | b , c |
| AV-17 | M/26 | Chest | a |
| AV-18 | F/ 23 | Face | c |
| AV-19 | M/20 | Face | a, b, c |
| AV-20 | M/17 | Face | a, b |
| AV-21 | M/21 | Face | a, b, c |
| AV-22 | F/18 | Back | a |
| AV-23 | F/16 | Face | a , c |
| AV-24 | F/20 | Face | b |
| AV-25 | M/20 | Chest | b, c |
| AV-26 | F/20 | Face | a |
| AV-27 | M/23 | Back | a |
| AV-28 | F/29 | Face | a, b, c |
| AV-29 | F/19 | Chest | a |
| AV-30 | M/26 | Back | a |
| AV-31 | M/19 | Chest | b |
| AV-32 | F/20 | Face | a, b, c |
| AV-33 | M/23 | Face | a, b |
| AV-34 | F/18 | Face | b |
| AV-35 | F/24 | Face | a |
| AV-36 | F/16 | Chest | b |
| AV-37 | M/25 | Face | b, c |
| AV-38 | F/24 | Back | a |
| AV-39 | F/19 | Chest | a, c |
| AV-40 | F/26 | Face | b |
| AV-41 | M/30 | Face | a |
| AV-42 | F/17 | Chest | a, b |
| AV-43 | F/28 | Face | b. c |
| AV-44 | M/16 | Face | a, b |
| AV-45 | M/26 | Chest | a, b, c |
| AV-46 | M/19 | Face | c |

F: Female; M: Male; a: *Staphylococcus aureus*; b: *Staphylococcus epidermidis;* c: *Micrococcus* spp

The results showed that between 70-75% of subjects in the age group of 18-29 years had acne, suggesting that hormonal influence of that particular age group play major role in causation of acne and this value is similar to the values reported by Asima *et al*., (2011). The occurrence of acne is more in females than in male in this study and this is contrary to the reports of Asima *et al*., (2011), which suggested no significant difference in occurrence of acne in males and females.

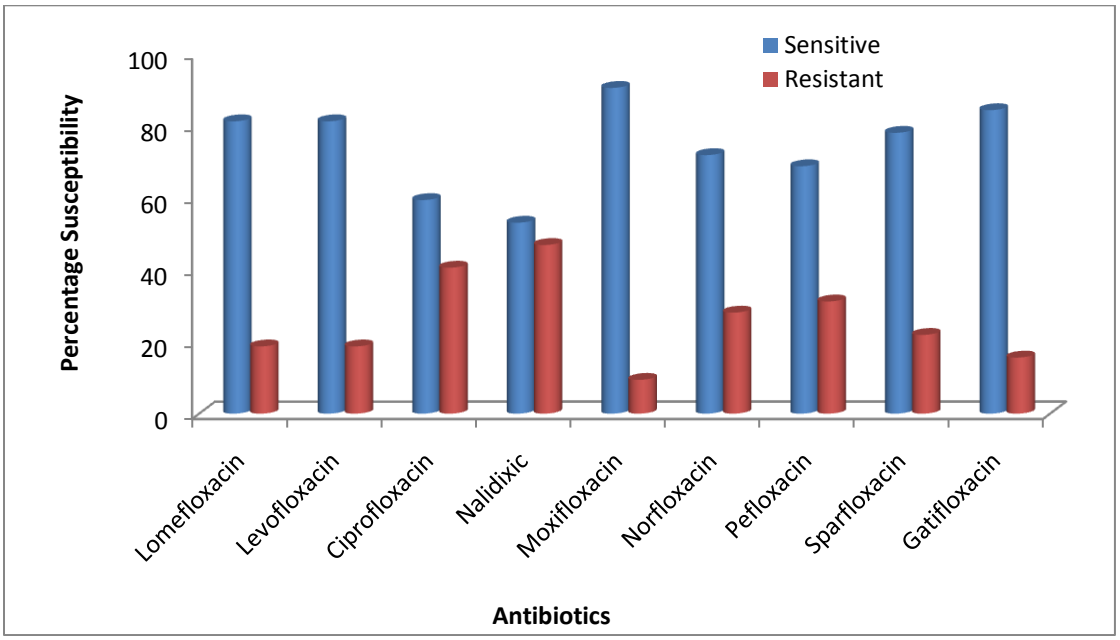


**Fig. 1.** Comparative Percentage of Antibiotic Susceptibility Profiles Between *Staphylococcus aureus* (n=32).

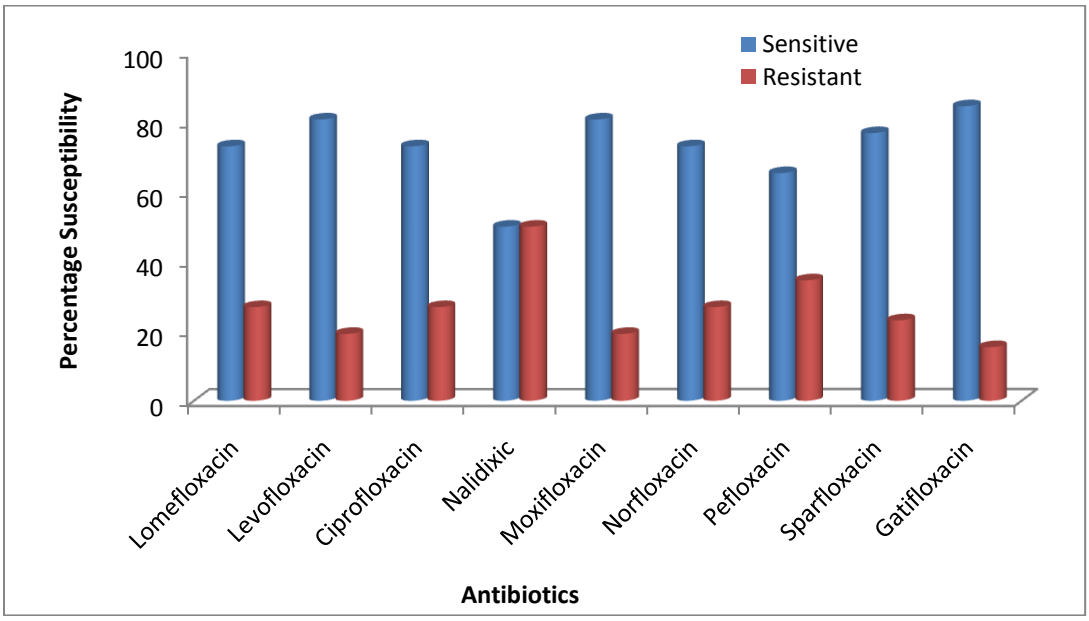


**Fig. 2.** Comparative Percentage of Antibiotic Susceptibility Profiles Between *Staphylococcus epidermidis* (n=26).

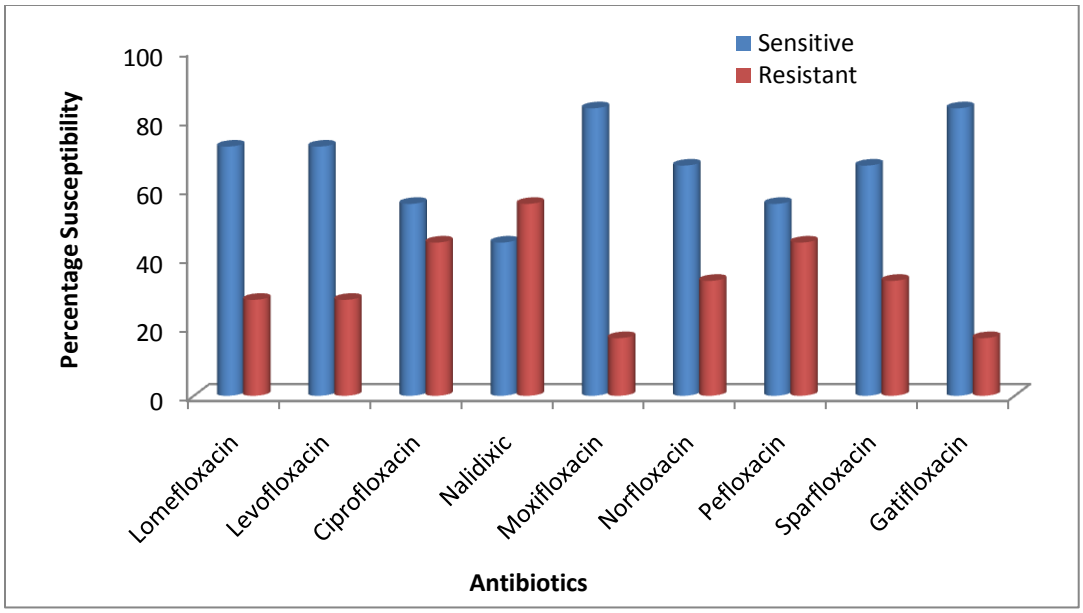


**Fig. 3.** Comparative Percentage of Antibiotic Susceptibility Profiles Between *Micrococcus* spp (n=18).

Deoxyribonucleases (DNases) produced by micro-organisms are extracellular endonucleases that cleave DNA, yielding a high concentration of oligonucleotide (Talaro and Talaro, 1996; Prescott *et al*., 2011). The Gram positive aerobic bacteria isolated from acne vulgaris showed DNase production ranging from 2 (11.1%) in *Micrococcus* spp to 26 (81.2%) in *S. aureus*. The production of DNase by *S. aureus* isolated from acne vulgaris in this study is in concordance with Finegold and Baron (1986). Prescott *et al*. (2008) also revealed that one of the virulence factors produced by *S. aureus* is DNase.

During the last 20 years, the number of topical and systemic drugs for the treatment of acne vulgaris has been enriched (Gollnick and Krautheim, 2003). The indiscriminate use of antibiotics whether topically or orally has raised concerns globally about the development, spread of resistant organisms and the fears about resulting antibiotic resistance. Fluoroquinolones are a family of potent synthetic broad spectrum antibiotics or antimicrobial agents, usually administered orally or sometimes intravenously for treatment of selected community acquired, nosocomial or serious infections (Nelson *et al.,* 2007). The targets of flouroquinolones activity are the bacterial DNA gyrase and topoisomerase IV enzymes essential for DNA replication and transcription (Nelson *et al.,* 2007; Akinjogunla and Eghafona, 2011). The *S. aureus, S. epidermidis* and *Micrococcus* spp. obtained were susceptible to the fluoroquinolones such as ciprofloxacin, moxifloxacin, ofloxacin and this is in agreement with Pietro (2006). Ciprofloxacin is an antibiotic that treat the bacteria around the follicle, which often leads to cyst-like acne. Ciprofloxacins also work by reducing chemicals produced by white blood cells, or by reducing the concentration of fat that contribute to inflammation (Pietro, 2006).

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