

Antibiograms of *Staphylococcus aureus* and *Pseudomonas aeruginosa* which Colonize the Conjunctiva of HIV/AIDS Patients in Relation to their CD4 Counts

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Abstract

One hundred (100) conjunctival swabs were collected from Enzyme-linked Immunosorbent Assay (ELISA)-confirmed HIV/AIDS seropositive patients who were referred to the HIV/AIDS laboratory of the University of Benin Teaching Hospital, Nigeria for routine CD4 count check. Swabs were bacteriologically processed and bacterial strains obtained from CD4 count groups were subjected to invitro antibiotic testing by disc diffusion technique in relation to their CD4 groups. Thirty four (34.0%) patients had CD4 counts within group 1 (<500 cells/ μ l), 36(36.0%) in group 2 (200 cells/ μ l) and 30(30.0%) in group 3 (>200 cell/ μ l). While there was no bacterial growth after 48hrs incubation recorded for group 1, only 5(13.9%) samples yielded growth of *Staphylococcus aureus* for group 2 with 31(86.1%) yielding no bacterial growth. All group 3 samples yielded profuse growth of which 11(36.7%) yielded *Pseudomonas aeruginosa* and 19(63.3%) yielded *Staph aureus*. *Staphylococcus aureus* strains isolated from the 19(63.3%) group 3 samples showed invitro sensitivity reactions to ten antibiotics as follows: zinnat (57.9%) pefloxacin (55.3%), rocephin (51.3%) and gentamycin (50.0%). The others recorded less than 50% sensitivity. Multiple resistance to 6(60%) of antibiotics used was shown by 6(31.6%) out of the 19 strains. The antibiogram of the 11(36.7%) *Pseudomonas aeruginosa* strains showed ciprofloxacin (52.3%) and gentamycin (50.0%) reaction. The remaining eight recorded less than 50% sensitivity of which, 4(36.4%) *P. aeruginosa* strains showed multiple resistance to 50% of antibiotics used. The 5(13.9%) group 2 *Staph. aureus* strains showed invitro antibiotic susceptibility reactions as follows: pefloxacin (65.0%), gentamycin (60.0%), rocephin (55.0%), ciprofloxacin (55.0%) and streptomycin (50.0%). Others recorded less than 50% reaction and none of the strains in this group recorded multiple resistances. Unpaired t-test analysis showed that whereas the differences in susceptibilities of both organisms to ofloxacin, pefloxacin, gentamycin, ampicillin, zinnat, rocephin, ciprofloxacin and sperfloxacin were insignificant ($P>0.05$), differences in susceptibilities to streptomycin was highly significant ($P<0.01$) and significant to augmentin ($P<0.05$).

Keywords: HIV, CD4 counts, *Staph. aureus*, *P. aeruginosa*, Colonize, Antibiograms

1. Introduction

The clinical manifestation of HIV secondary infections in developing countries including Nigeria shows a high prevalence of infections of the eyes, skin, among others (Akolo and Ukoli, 2005). The Human T-lymphotropic virus Type III (HTLV-III) has been found in tears thus indicating the presence of free virus in

tears. Retroviral patients in their immune-compromised state would have a wider range of bacterial organisms present in their eyes. These organisms include *Staphylococcus* spp and *Pseudomonas* spp among others. *Staphylococcus* and *Pseudomonas* organisms are opportunistic pathogens in humans which can infect the eyes through contaminated fingers/contact lenses. *Pseudomonas aeruginosa* has emerged as one of the most common causes of gram negative bacteraemia with possible dissemination to the eyes (Adeleye *et al.*, 2008; Hart *et al.*, 2000).

The presence of bacteria in the eye could lead to bacterial retinitis, conjunctivitis, keratitis, neonatal ophthalmia, hordeolum (swelling of the eyelid due to infection of the glands), and blepharitis (Yasuyuki and Ben, 2005). The complete tear fluid (secreted by the lachrymal gland) contains antibacterial enzymes like lysozyme which can kill commonly occurring micro organisms like *Staphylococci* and *Streptococci*. The tear film is the natural eye defense mechanism and is made up of mucin layer, aqueous layer and lipid layer. The aqueous layer contains antimicrobial factors such as lactoferrin, lysozyme, IgA and proteins such as beta lysins. All these help to protect the eye and the ocular surface.

The ocular surface is constantly exposed to a wide array of microorganisms and therefore, a combination of mechanical, anatomical and immunological defense mechanisms have evolved to protect the outer eye (Akpek and Gottsch, 2003). These defense mechanisms are usually overwhelmed in immune-compromised individuals (such as HIV/AIDS patients), individuals on corticosteroid therapy, adverse environmental changes and in debilitating disease conditions such as diabetes mellitus (Chiegbu *et al.*, 2003). An outcome of an immune-compromised HIV seropositive patient is direct effect on T-lymphocytes called CD4 cells.

The CD4 cells (also known as T-cells) are an important part of the immune system. People with AIDS (full blown) have fewer CD4 cells compared to seronegative healthy individuals (Adeleye *et al.*, 2010). Different people with AIDS may possess different values of CD4 cells. Those with higher CD4 counts usually feel better and are less likely to go down with opportunistic infections. Hence, a CD4 count assay is used to monitor the effectiveness of a course of treatment. If a particular anti-retroviral drug (medication) raises the level of CD4 count (from what it was before treatment), it means by interpretation, that the drug is working. Normal adult CD4 count reference varies between 500 –1500cell/mm³ whole blood with approximately 400 – 1200/mm³ for men and 500 -160/mm³ for women (CDC, 1993).

In addition, the CD4 T-cell values fluctuate with the time the assay is done. Fatigue and stress may also influence CD4 count (Ebona *et al.*, 2007). It is recommended that all patients' blood samples should be collected at the same daytime for each CD4 test and the same laboratory should be used to analyze all samples (Giorgis *et al.*, 2007). Other factors that can affect (increase) CD4 counts are infections (which naturally will increase white cell and lymphocyte counts), seasonal and diurnal variation, surgery, viral infections, tuberculosis and splenectomy (Giorgis *et al.*, 2007). Factors that can decrease CD4 counts are corticosteroid therapy, cancer therapy etc. Sex, race, psychological stress and physical stress have minimal effect on CD4 count. Prescott (1990), in a study, stated that *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* were found to adhere to corneal epithelium significantly better than other species that were tested for adhesion to corneal epithelium. This result suggested that the frequency with which certain bacteria cause corneal ulceration may be related to the ability of those organisms to adhere to corneal epithelium.

Moss *et al.* (2002) in a related study, associated *Staphylococcus aureus* colonization of nasal epithelium with CD4 T-cell count among HIV seropositive patients in a poor urban community ((P<0.05). According to their report, *Staph. aureus* colonization rate among HIV positive individuals increased as CD4 count decreased. Patients with CD4 counts below 200 had greater rates of nasal colonization than those with higher CD4 counts. It is expected as reported by Moss *et al.* (2002) that there will be stronger nasal colonization of bacteria particularly *Staph. aureus*. This stronger adhesion may lead to difficulties in antibiotic treatment of nasal infections due to these bacterial organisms.

According to Yasuyuki and Ben. (2005). *Staph. aureus* and *P. aeruginosa* are constantly linked with corneal infections in HIV patients. Practitioners may encounter difficulty in treating such infections owing to various immune-compromised stages HIV patients may be. This may or may not have direct relationship with viral load as against CD4 count. The aim of this work therefore, is to investigate the antibiograms of *Staphylococcus aureus* and *Pseudomonas aeruginosa* which colonize the conjunctiva of HIV/AIDS patients in relation to their CD4 counts with the following objectives: 1. Isolate *Staph. aureus* and *Pseudomonas aeruginosa* from the conjunctiva of HIV/AIDS seropositive patients taking note of their CD4 T-cell counts, 2. Establish antibiotic susceptibilities of these bacteria so as to determine if the susceptibility is due to their occurrence in relation to the CD4 counts of the

patients from whose eyes they are isolated. The idea is to facilitate the treatment of HIV/AIDS patients presenting with any eye disease using their CD4 counts as a guide.

2. Materials and methods

With informed consent and approval obtained from the ethical committee of the hospital management, 100 eye (conjunctival) swabs were collected from Enzyme-linked Immunosorbent Assay (ELISA) – confirmed HIV/AIDS seropositive patients who were referred to the HIV/AIDS laboratory of the University of Benin Teaching Hospital, Nigeria for routine CD4 count check (to monitor the progress of the disease and response to anti – retroviral therapy). Samples were collected between January to March, 2009.

Swabs were neatly collected (by retracting the lids carefully and swabbing through with sterile swab sticks) after blood samples had been withdrawn for CD4 T-cell counts by laboratory personnel. Patients' full data were noted (with proper labeling) in order to match culture results with CD4 counts. CD4 count results were collated and grouped into groups 1-3 according to Centre for Disease Control and Prevention⁹ scheme. The swab samples were coded 1-100 with names of patients written by each code. Only CD4 counts reported within 3 months (period of sample collection) were collated and used.

Swabs were cultured aseptically on sterile MacConkey, Blood and Mannitol salt agar plates and incubated at 37°C for 24 hours. Pure (axenic) isolates/strains were obtained and stocked on sterile nutrient agar slants. Gram staining was also done and results recorded.

Pure isolates were identified culturally, morphologically, biochemically and by sugar fermentation according to schemes provided by Cowan and Steel (1993) and Cullimore (2000). All catalase positive, coagulase positive colonies, gram positive cocci in clusters, glucose positive, mannitol positive (characteristic of *Staphylococcus aureus*) and all citrate positive, oxidase positive colonies (with gray-greenish pigmentation), short gram negative rods in singles (characteristic of *Pseudomonas aeruginosa*) were stocked on sterile agar slants for further use. Stocked pure cultures (strains) were then subjected to invitro antibiotic testing.

2.1 Invitro antibiotic testing

This was done according to the modified disc diffusion technique of Bauer *et al.* (1997). Sterile nutrient agar plates and peptone water prepared and dispensed into bijou bottles were prepared in accordance with manufacturer's instructions. An inoculum of the stock culture of each strain was subcultured into sterile peptone water (in bijou bottles) and incubated on the bench for 2-3 hours. A sterile peptone water bottle (which was not subcultured) was used as control. All steps were carried out with proper labeling.

Sterile nutrient agar plates were arranged and labeled for the strains of each isolate with one reserved as control. The plates were flooded with the liquid culture in the bijou bottles and the control bijou bottle was equally used to flood the control plate. The excess liquid culture was drained off the plates. Using well sterilized pair of forceps, commercially obtained gram positive and gram negative multidrug discs were impregnated on the flooded agar surface. While gram positive discs were used for *Staph aureus* strains, gram negative discs were used for *Pseudomonas aeruginosa*. The control plate was divided into two halves. On one half, gram positive discs were impregnated and gram negative discs on the other.

All plates were then incubated aerobically at 37°C for 24 hours. Results were interpreted according to the National Committee for Clinical Laboratory Standards.

3. Results

Out of the 100 blood samples assayed for CD4 counts, 34 (34.0%) had their counts within group 1 (>500 cells/μl) range, 36 (36.0%) within group 2 (200-499 cells/μl) range and 30 (30.0%) in the group 3 (< 200 cells/ μl) range (Table 1).

Bacteriological (cultural) results showed that all samples/patients in CD4 count group 1 yielded no bacterial growth after incubation. In the case of CD4 count group 2, whereas only five samples yielded growth of *Staphylococcus aureus*, the others did not yield any bacterial growth. All samples placed into group 3 yielded profuse growth of which 11 (11.0%) yielded *Pseudomonas aeruginosa* while 19 (19.0%) yielded *Staph. aureus*. On the whole, whereas 11 (11.0%) samples yielded profuse growth of *Pseudomonas aeruginosa*, 24 (24.0%) yielded *Staph. aureus* (Table 2).

Staph. aureus strains isolated from 19 group 3 samples (Table 2) were subjected (sensitivity testing) invitro to ten different antibiotics which included pefloxacin, gentamycin, ampiclox, zinnat (cefuroxime), ampicillin, rocephine (ceftriaxone), streptomycin, ciprofloxacin, septrin (clotrimoxazole) and erythromycin. The overall sensitivity pattern showed zinnat (57.9%), pefloxacin (55.30%), rocephine (51.3%), and gentamycin (50.0%) in

that decreasing order of sensitivity. The others which recorded less than 50% sensitivity were ciprofloxacin (47.4%), streptomycin (43.4%), septrin (26.4%), ampicillin (22.4%), erythromycin (22.4%) and ampiclox (7.9%). Of the 19 strains of *Staph. aureus*, 6 (31.6%) showed multiple resistance to 6(60%) of antibiotics used (Table 3).

Eleven samples which recorded CD4 count of >200cells/ μ l yielded 11 *Pseudomonas aeruginosa* strains. Their invitro antibiotic sensitivity results showed ciprofloxacin (52.3%) and gentamycin (50.0%) in that decreasing order. Susceptibilities of less than 50% were recorded for pefloxacin (49.5%), ofloxacin (43.2%), ampicillin (34.1%), streptomycin (29.6%), sperfloxacin (29.6%), septrin (27.3%), chloramphenicol (25.0%) and augmentin (22.7%) in that decreasing order (Table 4). Of the 11 strains, 4 (36.4%) showed multiple resistance to 6(60%) of antibiotics used.

Five samples from five patients who recorded 200-499 cells/ μ l CD4 count yielded five strains of *Staph. aureus* to which the drugs used in Table 1 were subjected. Their invitro sensitivity reactions were pefloxacin (65.0%), gentamycin (60.0%) rocephine (55.0%), ciprofloxacin (55.0%) and streptomycin (50.0%) in that decreasing order (Table 5). Sensitivities of less than 50% were recorded for zinnat (45.0%) septrin (35.0%), erythromycin (35.0%), ampicillin (30.0%) and ampiclox (25.0%) in that descending order (Table 5). There was no record of multiple resistance against drugs used for any of the strains.

The differences in susceptibilities between *Staph. aureus* and *Pseudomonas aeruginosa* to each of the antibiotics applied to both organisms were statistically analyzed using an unpaired t-test. As shown in Table 6, whereas the differences in susceptibilities of both organisms to ofloxacin, pefloxacin, gentamycin ampicillin, zinnat, rocephine, ciprofloxacin and sperfloxacin were insignificant ($P>0.05$), differences in susceptibilities to streptomycin was highly significant ($P<0.01$) and significant to augmentin ($P<0.05$).

4. Discussion

All patients used for the study were confirmed by Enzyme linked Immunosorbent Assay (ELISA) to be HIV seropositive. Patients may have been at various stages of advancement or progression of the disease. This may explain why 34 (34.0%) of them recorded a CD4 count greater than 500 cells/ μ l (Table 1). It may be that this group of patients recently got infected or recently became seropositive. The highest number of patients (36) and representing 36.0% of sampled population fell into the bracket of 200-499cells/ μ l or group 2 (Table 1). Here, CD4 counts have begun to drop suggesting progression of disease in terms of replication of the retrovirus inside T cells with their eventual damage and depletion. Patients in group 3 which represented 30% of samples recorded sharp drop in CD4 counts. There is likely to be further progression of disease giving room to opportunistic infections including bacterial infection (Ebana *et al.*, 2007).

According to CDC (1993), different people with AIDS possess varying values of CD4 T-cells and those with higher CD4 counts usually feel better and are less likely to go down with opportunistic infections. Biswas *et al.* (2000) reported that the pattern of ocular manifestations in HIV/AIDS patients observed in different studies seem to suggest that it is related to the CD4 counts. Related to this, is the finding of Copeland (2005) which stated that some sight threatening eye diseases in AIDS patients occur at late stage in the disease when the immunity has been severely compromised and with a CD4 count less than 100/ mm^3 .

The growth and isolation of 11 strains of *Pseudomonas aeruginosa* and 19 strains of *Staphylococcus aureus* from all 30 (30.0%) samples of group 3 somewhat confirms the extent of immuno compromised condition of this group of patients. Group 1 samples did not yield any bacterial growth and this is probably due to their intact state of health (though recently infected probably as evidenced by a CD4 count equal to or greater than 500)Table 2. Hence, HIV seropositive patients with CD4 counts below 200 have far greater rates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* colonization compared to those with higher CD4 counts and this tends to agree with the report of Moss *et al.* (2002) which stated that bacterial colonization increases with decreasing CD4 counts. Findings in this study are also supported by Adeleye *et al.* (2010), Farrokh *et al.* (2008) and Goose (1998) who reported that due to the weakened defense (immune) systems of HIV/AIDS victims, they become hosts especially to opportunistic pathogens.

Staphylococcus aureus strains isolated from 19 patients who recorded CD4 T-cell counts of less than 200 showed varying susceptibility patterns (antibiograms) to pefloxacin, gentamycin, ampiclox, zinnat, ampicillin, septrin, rocephine, streptomycin, ciprofloxacin and erythromycin invitro (Table 3). This sensitivity pattern differed from that obtained by Shittu and Lin (2006). In decreasing order of sensitivity, the strains were most sensitive to zinnat (57.9%), pefloxacin (55.3%), rocephine (51.3%) and gentamycin (50.0%). Unfortunately, the first three (which are broad spectrum antibiotics) are expensive and unaffordable to majority of victims due to their low socio-economic status or low income.

Ciprofloxacin, streptomycin, septrin, erythromycin, and ampicillin recorded less than 50% sensitivity in that decreasing order suggesting their unsuitability for treatment of bacterial infections of the eye of HIV/AIDS victims having CD4 counts less than 200. Apart from ciprofloxacin, the others are relatively cheap and affordable. Their low sensitivity may be as a result of resistance due to constant indiscriminate use and abuse of the drugs. According to Rawstron (1999), bacteria that used to be curable with antibiotics are now resistant. A combination of gentamycin and ampicillin or gentamycin and ampiclox however, will produce synergistic effect (Ochei and Kolhatkar, 2008).

The antibiogram of *Pseudomonas aeruginosa* strains isolated from the eyes of patients having CD4 count of less than 200 (Table 4) was a little different. The strains were sensitive to ciprofloxacin (52.3%) and gentamycin (50.0%) in that order. Those that were less than 50.0% susceptible included pefloxacin (49.5%), ofloxacin (43.2%), ampicillin (34.1%), streptomycin (29.6%), sperfloxacin (29.6%), septrin (27.3%), chloramphenicol (25.0%) and augmentin (22.7%) (Table 4). Again, apart from gentamycin, the others like ciprofloxacin and pefloxacin are expensive and maybe unaffordable. However combinations of gentamycin and other antibiotics having less than 50% susceptibility may produce synergistic response to most stubborn eye bacterial diseases caused by *Pseudomonas aeruginosa* (Manfredi *et al.*, 2000). The high degree of sensitivity of *Pseudomonas aeruginosa* strains to ciprofloxacin is supported by Manfredi *et al.* (2000) who reported 100% susceptibility to ciprofloxacin.

Pefloxacin (65.0%), gentamycin (60.0%), rocephine (55.0%), ciprofloxacin (55.0%) and streptomycin (50.0%) in that order was the antibiotic sensitivity pattern of *Staphylococcus aureus* strains isolated from patients whose CD4 counts ranged from 200-499 cells/ μ l (Table 5). This result suggests pefloxacin as the drug of choice for this category of patients. Again, apart from gentamycin and streptomycin, the others are expensive particularly when a prolonged dose is prescribed to tackle a chronic eye disease. Hence, gentamycin can still be used as an affordable and effective alternative.

The unpaired t-test statistical analysis of susceptibility differs between *Staph. aureus* and *Pseudomonas aeruginosa* to each of the antibiotics applied to both organisms showed that whereas the differences in susceptibilities of both organisms to ofloxacin, pefloxacin, gentamycin, ampicillin, zinnat, rocephin, ciprofloxacin and sperfloxacin were insignificant ($P > 0.05$), differences in susceptibilities to streptomycin was highly significant ($P < 0.01$) and significant to augmentin ($P < 0.05$) Table 6. The import of this is that any of ofloxacin, pefloxacin, gentamycin, ampicillin, zinnat, rocephine, ciprofloxacin or sperfloxacin can be used to treat any ocular infections caused by either *Staph. aureus* or *Pseudomonas aeruginosa* depending on their degree of sensitivity, cheapness and availability. However streptomycin (1.89 ± 0.06) will be much more effective in curing eye (conjunctival) infections due to *Staph. aureus* than that due to *Pseudomonas aeruginosa* ($P < 0.01$). Similarly, augmentin (1.83 ± 0.17) followed the same trend ($P < 0.05$).

5. Conclusion

The results of this study suggest pefloxacin as drug of choice for treatment of eye co-infections by *Staph. aureus* suffered by HIV/AIDS patients having CD4 counts of 200 – 499 cells/ μ l. In terms of affordability, gentamycin can be used as an effective alternative. Results also proffer cefuroxime (zinnat) or ciprofloxacin as drug of choice for treating eye disease suffered by HIV/AIDS patients having CD4 counts of less than 200 cells/ μ l with gentamycin as a standby potent alternative.

Based on statistical comparison of susceptibility differences between *Staphylococcus aureus* and *Pseudomonas aeruginosa* to each antibiotic applied to both organisms, any of ofloxacin, pefloxacin, gentamycin, ampicillin, rocephine, or sperfloxacin can be used to treat any ocular infections caused by *Staphylococcus aureus* or *Pseudomonas aeruginosa* depending on their degree of sensitivity, cheapness and availability.

Combinations of gentamycin and any other antibiotics having less than 50% susceptibility may produce synergistic effect on stubborn eye bacterial diseases caused by any of the two pathogens. The combination of gentamycin with any of the less sensitive antibiotics in small and non-toxic concentrations is recommended for application in the manufacture of eye drop solutions. The sustained use of gentamycin eye drops without any form of adulteration should be encouraged

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Table 1. Distribution of patients/samples into CD4 Groups

Swab Sample Codes	Total (%)	CD4 Group (CDC, 1997)
3,6,10,11,12,13,15 17,20,21,25,31,32, 34,38,40,42,44,45 46,50,51, 52,53,54,56, 57,58,59,60,61,62, 63,64	34 (34.0%)	Group 1 >500 cells/ μ l
65,66,67,68,69,70,71,72, 73,74,75,76,77,78,79,80, 81,82,83,84,85,86,87,88, 89,90,91,92,93,94,95,96, 97,98,99,100	36 (36.0%)	Group 2 200 - 499 cells/ μ l
1,2,4,5,7,8,9,14,16,18, 19,22,23,24,26,27,28, 29,30,33,35,36,37,39,41, 43,47,48,49,55	30 (30.0%)	Group 3 < 200 cells/ μ l

Table 2. Bacterial Colonization of the Conjunctiva in Relation to CD4 counts

CD4 Group	Swab Sample Codes	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	No Growth
Group 1 (< 500 cells/ μ l)	3,6,10,11,12,13,15,17, 20,21,25,31,32,34,38, 40,42,44,45,46,50,52, 53,54,56,57,58,59,60, 61,62,63,64	NiL	NiL	34
Group 2 (200-499cells/ μ l)	65,66,67,68,69,70,71,72, 73,74,75,76,77,78,79,80, 81,82,83,84,85,86,87,88, 89,90,91,92,93,94,95,96, 97,98,99,100	NiL	5	31
Group 3 (> 200 cells/ μ l)	1,2,4,5,7,8,9,14,16,18, 19,22,23,24,26,27,28, 29,30,33,35,36,37,39,41, 43,47,48,49,55	11	19	30

Note:

Group 2 swab codes that yielded *Staph.aureus*: 69, 71, 85 and 100

Group 3 swab codes that yielded *Staph aureus*: 7,9,14,16,18,19,22,23,24,26,27,28,31,36,37,41,43,49,55

Group 3 swab codes that yielded *Pseud. aeruginosa*: 2,5,7,16,19,22,24,28,30,33,55

Table 3. Antibigram of *Staphylococcus aureus* strains isolated from patients with <200cells/ µl) CD4 T-cell counts

Swab Sample code Nos	PEF (%)	ANGN (%)	TIBAPX (%)	IOTICZ (%)	SAM (%)	USERO (%)	DST (%)	CPX (%)	SEP (%)	E (%)
22	25.0	25.0	25.0	50.0	25.0	25.0	25.0	50.0	25.0	25.0
23	50.0	50.0	R	25.0	25.0	25.0	25.0	50.0	R	25.0
24	50.0	50.0	R	50.0	25.0	50.0	50.0	50.0	50.0	25.0
26	75.0	50.0	R	50.0	25.0	50.0	25.0	50.0	25.0	25.0
27	75.0	50.0	25.0	50.0	25.0	50.0	25.0	25.0	25.0	25.0
28	R	25.0	R	25.0	R	25.0	25.0	R	25.0	R
29	25.0	25.0	R	50.0	R	50.0	50.0	50.0	25.0	25.0
30	75.0	50.0	25.0	75.0	50.0	75.0	25.0	50.0	25.0	25.0
33	75.0	50.0	R	75.0	R	50.0	25.0	25.0	25.0	R
35	75.0	75.0	R	50.0	R	75.0	50.0	75.0	R	R
36	75.0	50.0	R	50.0	R	50.0	50.0	50.0	25.0	25.0
37	50.0	50.0	50.0	75.0	50.0	75.0	75.0	75.0	75.0	75.0
39	75.0	75.0	R	75.0	R	50.0	50.0	75.0	50.0	50.0
41	75.0	50.0	R	75.0	50.0	75.0	50.0	50.0	R	R
43	75.0	25.0	25.0	50.0	25.0	50.0	25.0	25.0	25.0	25.0
47	50.0	25.0	R	75.0	50.0	50.0	75.0	50.0	R	25.0
48	50.0	75.0	R	75.0	50.0	50.0	50.0	50.0	R	R
49	R	75.0	R	75.0	R	50.0	50.0	75.0	50.0	25.0
55	75.0	75.0	R	50.0	25.0	50.0	50.0	25.0	25.0	25.0
Total 19	1050	950.0	150.0	1100	425.0	975.0	825	900.0	475.0	425.0
Average	55.3%	50.0%	7.9%	57.9%	22.4%	51.3%	43.4%	47.4%	26.4%	22.4%

PEF=Pefloxacin, GN=Gentamycin, APX=Ampiclox, Z=Zinnat, AM=Ampicillin, RO=Rocephin, ST=Streptomycin, CPX=Ciprofloxacin, SEP=Seprin, E=Erythromycin

Table 4. Antibigram of *Pseudomonas aeruginosa* strains isolated from patients having < 200 (cells/ µl) CD4 counts

Swab Sample codes	OFX (%)	PEF (%)	GN (%)	AMP (%)	CPX (%)	ST (%)	SEP (%)	CHL (%)	SP (%)	AU (%)
1	25.0	R	R	25.0	50.0	R	R	R	25.0	R
2	75.0	75.0	50.0	50.0	75.0	75.0	R	25.0	50.0	25.0
4	25.0	50.0	75.0	25.0	25.0	R	25.0	R	R	25.0
5	50.0	50.0	50.0	50.0	50.0	25.0	25.0	25.0	25.0	25.0
7	75.0	75.0	75.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
8	50.0	50.0	50.0	25.0	50.0	25.0	25.0	25.0	25.0	R
9	50.0	50.0	50.0	25.0	50.0	50.0	50.0	25.0	25.0	R
14	25.0	25.0	50.0	R	50.0	R	25.0	25.0	25.0	R
16	R	70.0	25.0	25.0	50.0	R	R	R	R	25.0
18	50.0	50.0	75.0	50.0	75.0	50.0	50.0	50.0	50.0	50.0
19	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Total 11	475.0	550.0	550.0	375.0	575.0	325.0	300.0	275	325.0	250.0
Average	43.2%	49.5%	50.0%	34.1%	52.3%	29.6%	27.3%	25.0%	29.6%	22.7%

PEF=Pefloxacin, GN=Gentamycin, AM=Ampicillin, OFX=Ofloxacin, ST=Streptomycin, CPX=Ciprofloxacin, SEP=Seprin, CHL=Chloramphenicol, SP=Sperfloxacin, AU=Augmentin

Table 5. Antibigram of *Staph. aureus* strains isolated from patients having 200-499 cells/ μ l CD4 T-cell counts

Swab Sample code	PEF (%)	GN (%)	APX (%)	Z (%)	AM (%)	RO (%)	ST (%)	CPX (%)	SEP (%)	E (%)
65	75.0	75.0	25.0	25.0	25.0	75.0	50.0	75.0	25.0	50.0
66	75.0	50.0	25.0	50.0	25.0	50.0	50.0	50.0	25.0	25.0
67	50.0	50.0	25.0	25.0	25.0	25.0	50.0	25.0	50.0	25.0
68	50.0	50.0	25.0	50.0	25.0	50.0	25.0	50.0	25.0	25.0
69	75.0	75.0	25.0	75.0	50.0	75.0	75.0	75.0	50.0	50.0
Total 5	325.0	300.0	125.0	225.0	150.0	275.0	250.0	275.0	175.0	175.0
Average	65.0%	60.0%	25.0%	45.0%	30.0%	55.0%	50.0%	55.0%	35.0%	35.0%

PEF=Pefloxacin, GN=Gentamycin, APX=Ampiclox, Z=Zinnat, AM=Ampicillin, RO=Rocephin, ST=Streptomycin, CPX=Ciprofloxacin, SEP=Septrin, E=Erythromycin

Table 6. Unpaired t-test statistical Analysis of Susceptibility Differences in response to Antibiotics by *Staph. aureus* (n=24) and *Pseudo. aeruginosa* (n=11)

Antibiotics	<i>Staph. aureus</i> ($\bar{X} \pm S.E$)	<i>Pseudo. aeruginosa</i> ($\bar{X} \pm S.E$)	P-value
OFX	1.33 $\pm$ 0.67	1.89 $\pm$ 0.14	P > 0.05 (0.580)
PEF	2.06 $\pm$ 0.13	2.00 $\pm$ 0.12	P > 0.05 (0.776)
GN	2.04 $\pm$ 0.07	1.67 $\pm$ 0.32	P > 0.05 (0.128)
AM	1.26 $\pm$ 0.15	1.50 $\pm$ 0.20	P > 0.05(0.362)
Z	1.96 $\pm$ 0.10	2.00 $\pm$ 0.08	P > 0.05 (0.802)
*ST	1.89 $\pm$ 0.06	1.06 $\pm$ 0.35	P < 0.01 (0.002)
RO	1.39 $\pm$ 0.15	1.11 $\pm$ 0.29	P > 0.05 (0.350)
CPX	1.33 $\pm$ 0.67	1.06 $\pm$ 0.27	P > 0.05 (0.790)
SP	1.33 $\pm$ 0.67	1.44 $\pm$ 0.19	P > 0.05 (0.914)
*AU	1.83 $\pm$ 0.17	0.72 $\pm$ 0.29	P < 0.01 (0.0014)

25.0% = mildly sensitive

50.0% = moderately sensitive

75.0% above = markedly sensitive

R = Resistant, ST = streptomycin, RO = Rocephine, CPX = Ciprofloxacin, SEP = Septrin, E = Erythromycin, CHL = Chloramphenicol, GN = Gntamycin, APX = Ampiclox, AM = Ampicillin, SP = Sperfloxacin, AU = Augmentin, Z = Zinnat, OFX = Ofloxacin (Tarivid), PEF = pefloxacin, AMX = Amoxicillin Significant Results