

Resistance of Nosocomial Bacterial Strains to Commonly Used Antibiotics and their Sensitivity to some Cameroonian Medicinal Plant Preparations.

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ABSTRACT

An epidemiological study of the resistance of some nosocomial bacterial strains to commonly-used antibiotics was carried out in the Bafang Ad-Lucem Hospital, West Province of Cameroon, from January 2001 to April 2002. One hundred and ten swab samples collected from surgical septic wounds were analysed. Thirty five per cent of *Pseudomonas* isolates, 21 % of *Klebsiella*, 7 % of *Protens* and 100% of *Alcaligenes* isolates were found to be multiresistant to antibiotics. Resistance to Gentamicin increased progressively with time, and was highest among the *Klebsiella* isolates. The inhibitory and bactericidal activities of extracts and purified compounds from seven medicinal plants locally used against wounds and ulcers were tested against selected multiresistant strains of *Pseudomonas aeruginosa*, *Alcaligenes* sp., and *Klebsiella pneumoniae*. The three bacterial strains were all found to be sensitive to aqueous stem bark extracts of *Parkia biglobosa*, *Alstonia boonei*, *Voacanga africana*, the fruit extract of *Aframomum melegueta*, the leaf methanol extract from *Ocimum suave*, and TN (a purified compound prepared from the fruit of *Voacanga africana* and identified with the Meyer and FeCl₃ tests as an alkaloid devoid of phenolic groups). The methanol extract of *Bidens pilosa* leaves was effective only against *Alcaligenes* sp.. PAL (7,8,-dihydro-8-hydroxypalmitine) a protoberberine alkaloid from *Enantia chlorantha* was not effective. The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration values for crude extracts ranged from 1.30 – 12.50 mg/ml and 5.20 – 19.40 mg/ml, respectively. Corresponding values for TN ranged from 3.13 – 25 mg/ml and 12.50 – 25 mg/ml. The results demonstrate the need for wider studies to establish nation-wide nosocomial bacterial sensitivity patterns, as well as the search for alternative drugs against multiresistant pathogens.

Keywords: Nosocomial infections, epidemiology, multi-drug resistance, medicinal plant preparations.

RÉSUMÉ

Une étude épidémiologique de la résistance des souches nosocomiales aux antibiotiques usuels a été menée à l'Hôpital Ad-Lucem de Bafang, Province de l'Ouest, Cameroun, entre janvier 2001 et avril 2002. Cent dix échantillons prélevés des plaies chirurgicales infectées ont été analysés et les antibiogrammes faits sur les germes isolés. Trente cinq pour cent de *Pseudomonas* spp., 21% de *Klebsiella* spp., 7% de *Protens* spp. et les deux seuls (100%) isolats d'*Alcaligenes* spp. ont été multirésistants. Au cours du temps, une augmentation progressive de la prévalence de résistances à la Gentamicine, antibiotique de choix commun aux germes étudiés, a été observée. Cette augmentation a été plus élevée parmi les souches *Klebsiella* sp. L'activité inhibitrice et bactéricide de certaines préparations à base des plantes médicinales utilisées localement dans le traitement des plaies et des ulcères a été testée sur une souche multirésistante de *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* et d'*Alcaligenes* spp.. Toutes ces souches ont été sensibles aux extraits aqueux de fruits d'*Aframomum melegueta*, des écorces de *Parkia biglobosa*, *Alstonia boonei*, *Voacanga africana*, ainsi que de l'extrait au méthanol de *Ocimum suave* et de TN (un alcaloïde purifié des fruits de *Voacanga africana* et identifié par les tests de Meyer de FeCl₃ comme étant dépourvu de groupements phénoliques). L'extrait au méthanol de *Bidens pilosa* n'a été actif que sur l'*Alcaligenes* sp. PAL (7,8,-dihydro-8-hydroxypalmitine), un alcaloïde de type protoberberine préparé à partir des écorces d'*Enantia chlorantha* n'avait aucune action sur les souches testées. Les valeurs de Concentration Minimale Inhibitrice et Concentration Minimale Bactéricide pour les extraits brutes variaient de 1.30 – 12.50 mg/ml et 5.20 – 19.40 mg/ml, respectivement. Les valeurs correspondantes pour TN variaient de 3.130 – 25 mg/ml et 12.50 – 25 mg/ml. Les résultats obtenus indiquent la nécessité d'une étude élargie à d'autres sites afin d'établir une carte nationale de la sensibilité des souches nosocomiales. Des études supplémentaires sont également nécessaires pour confirmer l'utilisation de ces plantes comme sources alternatives de nouveaux médicaments contre les souches multirésistantes.

Mots clés : Infections nosocomiales, épidémiologie, Résistance aux antibiotiques, plantes médicinales.

INTRODUCTION

The well-known reputation of hospitals as a source of infection has been documented since the mid-19th century, when the lives of patients under surgical operation were threatened by the possible acquisition of gangrene or puerperal sepsis (Simpson, 1992). Nosocomial infections (NIs) or hospital-acquired infections are infections developed by hospitalised patients as a consequence of their stay in the hospital (Brock and Medigan, 1988). NIs can be of endogenous origin, i.e. where the infections are due to the patients' own germs adhering to their skin and clothing, or of exogenous origin, where infections are due to cross (patient-to-patient) contamination especially through germ transfer from contaminated hospital devices or by medical personnel. It is estimated that approximately 2 million nosocomial infections occur annually in the USA and 90,000 of the patients die as a result of these infections. Thus, nosocomial infections result in substantial mortality and morbidity, leading to invalidity and loss of production (Jarvis, 1998). In developing countries, the affected families are ruined as little or no social care is provided and the victims have to support the excess treatment charges.

About 60% of hospital-acquired infections are due to Gram-negative rods among which *Pseudomonas aeruginosa* (*P. aeruginosa*), *Proteus* spp, *Escherichia coli* (*E. coli*) and *Klebsiella* spp. are the most frequent, and to a lesser extent, *Alcaligenes* spp (more frequently involved in wound infections). *Staphylococcus aureus*, *Streptococcus* species and *Clostridium perfringens* contribute to 30% of the cases (Collee, 1992; Simpson, 1992).

A wide variety of nosocomial pathogens has developed resistance to many antibiotics including the newer and most potent ones. This has compromised the traditional therapeutic regimens, making the treatment of infected wounds more difficult and frequently more expensive (Tenover, 1995; 2001). In 35- 40% of NIs that occur, the pathogen is actually resistant to the best drug of choice (Jarvis, 1998). Monitoring and prevention of nosocomial infections is therefore of paramount importance in hospital policy. However, the purpose of any prevention and monitoring strategy is usually to reduce the rate of nosocomial infections to controllable levels, since prospects of complete eradication remain utopian (Avril and Carlet, 1998).

The present study was carried out in the Bafang Ad-Lucem Hospital, West Province, Cameroon. An epidemiological investigation of the resistance of nosocomial bacterial strains to commonly-used antibiotics was carried out. The nosocomial strains were isolated from septic wounds on hospitalised patients. The susceptibility of the pathogens to 15 commonly-used commercialised antibiotics was tested and isolates that were found to be sensitive to at most only one of the 15 drugs were considered to be multiresistant. The susceptibility of the most resistant strains (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Alcaligenes* sp.) to extracts and pure compounds from seven medicinal plants locally used against wounds and ulcers was tested. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the effective plant preparations were determined.

MATERIALS AND METHODS

2.1. EPIDEMIOLOGY OF BACTERIAL RESISTANCE TO ANTIBIOTICS

2.1.1. Patients and collection of samples

Patients were adults (above 15 years of age) hospitalised in the surgical ward of the Bafang Ad-Lucem Hospital following a surgical intervention, and having a septic wound resisting to normal wound dressing. Sterile cotton wool swabs were used to obtain samples by swabbing the suppurated area of the wound. To avoid contamination of the swab by normal skin flora, the surrounding skin area was first of all cleaned with "betadine". Each sample was prepared in duplicate; one was to be used for direct microscopy and the other for culture (Cheesbrough, 1985). Patients with fresh surgical wounds or fresh wounds from cuts and abscesses were excluded from the study. Samples were collected between January 2001 and April 2002. They were 110 in number and the germs of interest included *Pseudomonas* spp., *Klebsiella* spp., *Alcaligenes* spp and *Proteus* spp.

2.1.2. Isolation of the germs and antibiotic sensitivity test

Culture for isolation of the pathogens was performed routinely to seek not only the germs of interest but also other eventual pathogens in the sample. The standard method of wound sample treatment described by Cheesbrough (1985) was adopted. The isolated germs were identified

biochemically following the techniques described by Sydney and Hellen (1986).

Antibiotic sensitivity tests were done by disk diffusion techniques following "Sanofi Diagnostic Pasteur" (1993) and NCCLS (1997) standards. Each Isolate was tested against 15 commonly-used antibiotics. The data obtained were analysed to evaluate the prevalence of the germs, percentage resistance of the isolates to the various antibiotics used, the frequency of multiresistant isolates and the prevalence of resistance of the isolates to the various drugs of choice. The dynamics (progression) of resistance to Gentamicin, the cheapest drug of choice common to the three species of interest, was also studied.

2.1.3. Statistical analyses

The data obtained were analysed in order to evaluate the prevalence of the germs, the percentages of resistance of the isolates to the various antibiotics used, the prevalence of resistance of the isolates to the various drugs of choice and the dynamics of resistance to Gentamicin (i.e. the increase with time of the number of isolates resistant to Gentamicin). Statistical analysis of the dynamics of resistance to Gentamicin was done using the Spearman range test (Lecoutre and Tassi, 1987). Data were grouped in periods of four months, and analysed to obtain the progress of resistance with time. Following the Spearman range test, a positive correlation was obtained (with an error range of 5%) when the linear correlation coefficient ' r_s ' = 0.800.

2.2. SENSITIVITY OF MULTIRESTANT ISOLATES TO LOCAL MEDICINAL PLANT PREPARATIONS

2.2.1. Medicinal plant preparations.

All the plants studied were harvested and botanical identification was done at the Cameroon National Herbarium, Yaounde.

Aqueous extracts: The stem bark of *Voacanga africana* (voucher specimen No. HNC/1949) and *Alstonia boonei* (voucher specimen No. HNC/1943) were harvested around the Yaounde University I campus in April 2001. *Parkia biglobosa* (voucher specimen N° HNC/58980) stem bark was harvested in Maroua in December 2000. The fruits of *Aframomum melegueta*

(voucher specimen No. HNC/43123) were purchased from Bafang market in the same period. These plants were identified in the National Herbarium, Yaoundé. The plant parts were sun-dried until constant weight was attained and each ground to a powder using a blender mill. A decoction of each plant was made and the concentration determined as previously described (Boda *et al.*, 1998). These were used immediately or stored at 4°C.

Methanol extracts: The leaves of *Bidens pilosa* (voucher specimen No. HNC/58742) were harvested in November around the campus of the University of Yaoundé I and the methanol extract prepared as described by Tan *et al.* (2000a).

The leaves of *Ocimum. suave* (voucher specimen No. HNC/6077/6914) were harvested in August 2000 from the *Sabga* and *Wainama* hills of the Bamenda highlands. One kilogram of the dried leaf powder was subjected to cold extraction in 2 litres of methanol for 48 hours. The solution obtained was concentrated using a rotavapor, then re-dissolved in water to obtain the water-soluble fraction of the methanol extract. This was evaporated to dryness at 50°C in an air oven to obtain a brownish solid.

Purified compounds: TN is an alkaloid obtained from the 1:1 methylene chloride/methanol extract of the dried powder of the fruits of *Voacanga africana*. (Tan and Nyasse, 2000). Fresh mature fruits of *V. africana* were harvested in Yaoundé, sun dried and ground to a powder. The dried powder (5 kg) was extracted using a 1:1 mixture of methylene chloride/methanol (5 litres) and the solid matter (300 g) obtained after evaporation of the extraction solvent was fractionated by bioassay-guided procedure to obtain 5 g of the pure compound (TN). Using the Meyer and FeCl₃ tests, the compound was identified as an alkaloid not containing phenolic groups. Preliminary chemical and physical data suggested that the compound may correspond to tabersonine hydrochloride (m.p.192-195; [K]_D -307; elemental analysis: C₂₀H₂₆O₂N₂.HCl). The compound was code named TN and its detailed structural elucidation awaits publication.

PAL is a protoberberine alkaloid (7,8-dihydro-8-hydroxypalmatine) produced from the bark of *Enantia chlorantha* (voucher specimen No. 25918/

SRFCAM) The extraction, isolation and purification of the active compound were done as previously reported (Tan et al, 2000b). In brief, the stem bark methanol extract was evaporated till dryness and treated with a 10% H₂O/HCl solution to obtain a yellow solid precipitate. This was further extracted with methylene chloride and the fluffy solid material obtained after lyophilisation was chromatographed on silica gel using hexane/methylene chloride mixtures of increasing polarity. A further purification on silica gel column gave a solid that was recrystallised from hot methanol to yield the yellow pure compound (7, 8-dihydro-8-hydroxypalmitine), code named **PAL**. For a more detailed description of the characterisation of the compound, see Wafo *et al.* (1999).

2.2.2. Antimicrobial testing of plant preparations

Test organisms: They were all multiresistant strains isolated from septic wounds. They included isolates of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Alcaligenes* sp. They were all maintained on nutrient agar slopes at 4°C and sub-cultured in nutrient broth 24 hours prior to antimicrobial screening.

Standard inocula: A turbidity standard of 0.1 to 0.12 absorbance at 550 nm was prepared by diluting a 18 to 24 hour broth culture using sterile nutrient broth as diluent and as blank. This preparation was further serially diluted 10-fold to make a series of six 10-fold decreasing cell suspensions. One hundred microlitres (0.1 ml) from each dilution was used to inoculate a nutrient agar plate. After 24 hours of aerobic incubation at 37°C, the dilution that yielded a plate of semi-confluent growth was selected as the standard inoculum. To determine the cell suspension in the standard inoculum, the colony count of a plate in the series with evenly distributed and isolated colonies was done. The concentration (C) of the standard inoculum was determined as $C = (\text{count} \times 10^n) / 0.1$. 'C' is the concentration in Colony forming units per ml (CFU/ml); 'count' is the number of colonies counted; 'n' is the number of dilution steps that were taken to arrive at the standard inoculum (Boda, 1997). The cell suspensions of the various standard inocula were as follows: *P. aeruginosa* (1.1×10^5 CFU/ml), *K. pneumoniae* (1.21×10^5 CFU/ml), and *Alcaligenes* sp. (1.75×10^5 CFU/ml).

Antimicrobial tests: Aqueous extracts were first of all concentrated to make a concentration of 200

mg/ml by allowing 20 ml of extract to evaporate till dryness at 40°C. The dried plant material was harvested and re-dissolved with the corresponding amount of distilled water. For methanol extracts and purified compounds, solutions of 100 mg/ml were prepared using a (v/v) solution of 20% DMSO/0.25% Tween 80 as solvent. Mueller Hinton Agar (MHA) plates were inoculated with 0.1ml of standard inoculum and allowed to dry. Using the bold end of a sterile Pasteur pipette, 8 wells (equidistant to each other) were made on each agar plate and 60 µl of each of the 7 plant preparations were dropped into an allocated well. 60 µl of solvent was dropped into the remaining well to serve as the control. The extracts were allowed to dry by incubating the plate cover upturned for 1 to 2 hours before inverting (agar up). After 24 hours, the plant preparation that showed a visible clear zone of inhibition around the well was considered to be effective and was selected for further analyses.

MIC and MBC values were determined based on the method described by Collins and Lynes (1995), but with some modifications (Boda, 1997). Two-fold serially decreasing concentrations of the various plant preparations were prepared using a sterile v/v solution of 20 % DMSO/0.25 % Tween 80 as diluent. Two ml aliquots of the solution were dispensed aseptically (in duplicate) into sterile test tubes. Two ml of a double strength plant preparation-free Mueller Hinton Broth (MHB) were added to each of the test tubes. For each concentration, one of the tubes was inoculated with 0.1 ml of standard inoculum, while the other served as the negative control. A positive control broth, free from plant preparation and incorporated with 20 % DMSO/0.25 % Tween 80 was also inoculated. After 24 hours of incubation the culture was checked for turbidity (sign of growth), compared to the negative control tube. The MIC was considered to be the lowest plant preparation concentration that prevented the growth of the test organism.

For the MBC determination, 0.1 ml portions from all the tubes that did not show any sign of growth were sub-cultured on MHA plates containing no test plant product. The lowest concentration of plant preparation that did not produce growth after 24 hours of incubation of the plates was considered as the MBC.

RESULTS

EPIDEMIOLOGY OF RESISTANCE OF THE ISOLATES

Out of the 110 wound samples analysed within the set period of study, 34 isolates (44%) of *Pseudomonas* spp, 27 isolates (35%) of *Proteus* spp , 14 isolates (18%) of *Klebsiella* spp and 2 isolates (2%) of *Alcaligenes* spp were obtained. The number of multiresistant strains obtained were, respectively, 12/34 (35%), 3/27 (7%), 3/14 (21%) and 2/2 (100%) for *Pseudomonas*, *Proteus*, *Klebsiella* and *Alcaligenes* species. The prevalence of resistance to commonly used antibiotics among the isolates is shown in Table 1. *Pseudomonas* and *Klebsiella* isolates were found to be the most resistant, with prevalence of resistance greater than 50% recorded respectively against 11 and 9 out of 19 antibiotics used.

The sensitivity of *Proteus* species to antibiotics was relatively higher, with only 6 cases of high resistance obtained against amoxicillin, augmentin, cefoxitin, chloramphenicol, nalidixic acid and cotrimoxazole

(Table 1). A closer look at the activity of the various drugs of choice against the species studied revealed that only isolates of *Proteus* spp showed enough susceptibility to gentamicin, cefotaxime, cefuroxime, ciprofloxacin and perfloxacin, with resistance prevalence to these antibiotics not greater than 40 % (Table 2). The results obtained with gentamicin, cefoperazone, pefloxacin and ofloxacin on *Pseudomonas* sp. were disturbing, as prevalence of resistance to these antibiotics was not below 50 %. *Klebsiella* sp were the most resistant to the various drugs of choice as no resistance prevalence values of less than 40 % were obtained for this species.

Figure 1 shows the resistance dynamics to gentamicin, the cheapest drug of choice common to the three pathogenic species of interest. The Spearman range test revealed that the Linear Correlation coefficients 'r_s' for the three species were 0.800, 0.950 and 0.951, respectively, for *Pseudomonas* sp, *Proteus* sp. et *Klebsiella* sp.

The occurrence of resistant isolates among *Pseudomonas* species increased from 0 % at the beginning of the study to 75% (r_s = 0.800) at the end of the study. That of *Proteus* rose from 20% to

Table 1: Prevalence (%) of Resistance of Isolates to the various commercialised antibiotics

	<i>Pseudomonas spp.</i> (34 isolates)	<i>Proteus spp.</i> (27 isolates)	<i>Klebsiella spp.</i> (14 isolates)
Ampicillin	/	44	/
Amoxicillin	/	74	86
Augmentin	68	67	57
Cefoxitin	76	52	64
Cefuroxime	/	44	50
Cefotaxime	50	33	36
Netilmicin	70	48	50
Gentamicin	50	37	57
Doxycyclin	88	/	/
Tetracycline	85	/	/
Chloramphenicol	76	67	57
Lincomycin	82	/	57
Nalidixic acid	/	70	71
Cotrimoxazole	100	74	64
Pefloxacin	56	33	57
Norfloxacin	35	41	43
Ofloxacin	85	41	50
Ciprofloxacin	41	37	/
Cefoperazone	62	/	43

Key: / : Not tested

Table2: Prevalence (%) of the resistance of the pathogens to the various drugs of choice

	<i>Pseudomonas spp.</i> (34 isolates)	<i>Proteus spp.</i> (27 isolates)	<i>Klebsiella spp.</i> (14 isolates)
Gentamicin	50	37	57
Cefotaxime	/	33	57
Cefuroxime	/	44	50
Augmentin	/	67	/
Ciprofloxacin	41	37	/
Pefloxacin	56	33	57
Cefoperazone	62	/	43
Norfloxacin	35	/	43
Ofloxacin	85	/	50

Key:

/: not tested

50% ($r_s = 0.950$) and that of *Klebsiella spp* rose from 33 % to 100% ($r_s = 0.951$) during the same period.

ANTIMICROBIAL ACTIVITY OF LOCAL MEDICINAL PLANT PREPARATIONS

Screening studies: Out of a total number of eight plant preparations tested, seven were active against at least one of the test organisms, by showing an observable clear zone of inhibition around the well (Table 3). These included the extracts from *P. biglobosa*, *A. boonei*, *V. africana*, *A. melegueta*, *B. pilosa*, *O. suave*, and TN. PAL, the purified compound from *Enantia chlorantha* was not active. *Bidens pilosa* was active only against the *Alcaligenes sp.*, the organism noted to be the most sensitive to

the plant preparations.

Bactericidal and bacteriostatic activity: The activities of TN and the extract from *O. suave* on *K. pneumoniae* and that of the extract from *Bidens pilosa* on *Alcaligenes sp.* were only bacteriostatic since the corresponding tubes showing inhibition produced growth after subculture on test-compound-free agar plate. The various MIC values for crude extracts ranged from 1.30 – 12.50 mg/ml, with the lowest value (1.30 mg/ml) obtained with *P. biglobosa* stem bark extract on *Pseudomonas aeruginosa* and the highest (12.50 mg/ml) obtained with *O. suave* on *Klebsiella pneumoniae* (Table 3). The MIC values of TN ranged from 3.13 – 25 mg/

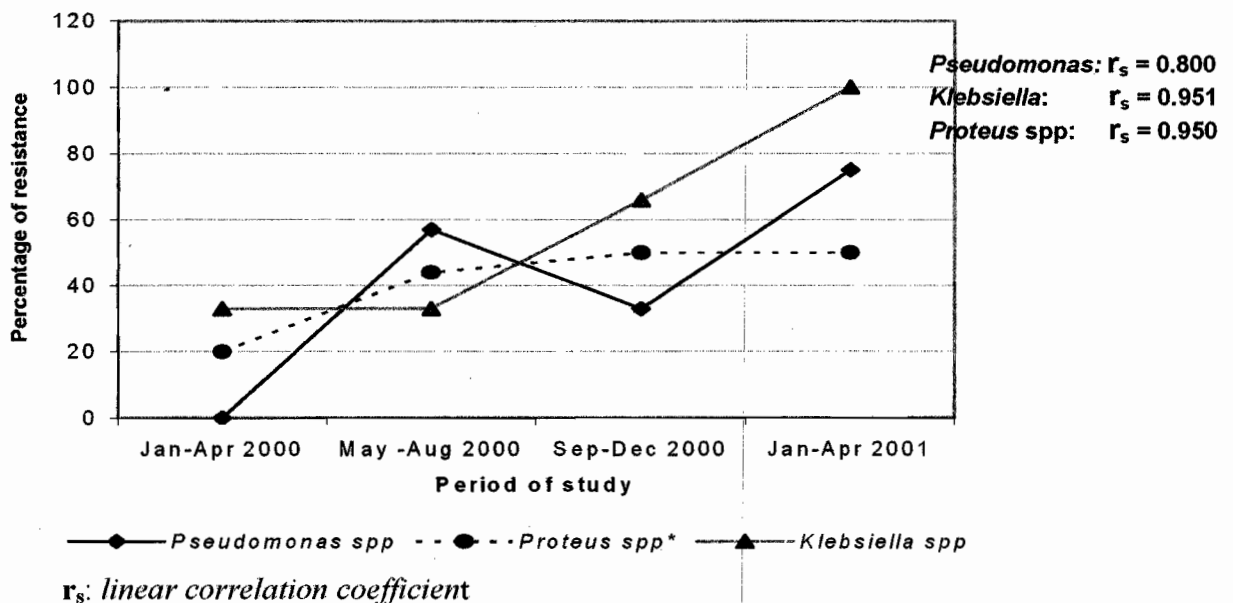


Fig. 1: Dynamics of resistance to Gentamicin.

Table 3: Minimum Inhibitory Concentrations and Minimum Bactericidal Concentrations (mg/ml) of plant preparations on test organisms.

	<i>P. aeruginosa</i> AP14401		<i>K. pneumoniae</i> AP8801		<i>Alcaligenes spp</i> AP15101	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>Aframomum melegueta</i>	3.00	6.00	3.00	12.00	1.50	6.00
<i>Alstonia boonei</i>	8.20	16.40	8.20	16.40	2.10	8.20
<i>Bidens pilosa</i>	—	—	—	—	6.25	A
<i>Ocimum suave</i>	3.125	12.50	12..50	A	3.13	12.50
<i>Parkia biglobosa</i>	1.30	10.40	2.60	5.20	2.60	5.20
<i>Voacanga africana</i>	9.70	19.40	4.85	9.70	4.85	9.70
TN	12.50	25.00	25.00	A	3.13	12.50
PAL	—	—	—	—	—	—

Key: A: Plant preparation only bacteriostatic but not bactericidal.

—: No antimicrobial activity observed

ml; the highest value was obtained on both *P. aeruginosa* and *K. pneumoniae* and the lowest value on *Alcaligenes* sp. The *Alcaligenes* sp. was highly sensitive to all the crude extracts, with all the MIC values obtained being less than or equal to 6.50 mg/ml compared with the purified compound TN.

As indicated by the graph of the level of inhibitory activity (1/MIC) presented in Figure 2, the values show that, the aqueous stem bark extract of *P. biglobosa* was most effective in inhibiting bacterial growth, especially on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The extract from the fruits of *A. melegueta* also had relatively high inhibitory effects (1/MIC > 0.3) on the test organisms especially on the *Alcaligenes* sp. (Figure 2). The stem

bark extracts of *A. boonei* and *V. africana* as well as the methanol extract of *O. suave* showed moderate inhibitory activities. TN and the methanol extract of *B. pilosa* were poorly active against *P. aeruginosa* and *K. pneumoniae*

The MBC values for crude extracts ranged from 5.20 to 19.40 mg/ml; the lowest value (5.20 mg/ml) was obtained with *P. biglobosa* stem bark aqueous extract on *K. pneumoniae* and *Alcaligenes* sp., while the highest (19.40 mg/ml) was obtained with *V. africana* stem bark extract on *Pseudomonas aeruginosa* (Table 3). The MBC values for TN on the test organisms were 12.50, 25.00 and 12.50 mg/ml respectively for *P. aeruginosa*, *K. pneumoniae* and *Alcaligenes* sp.

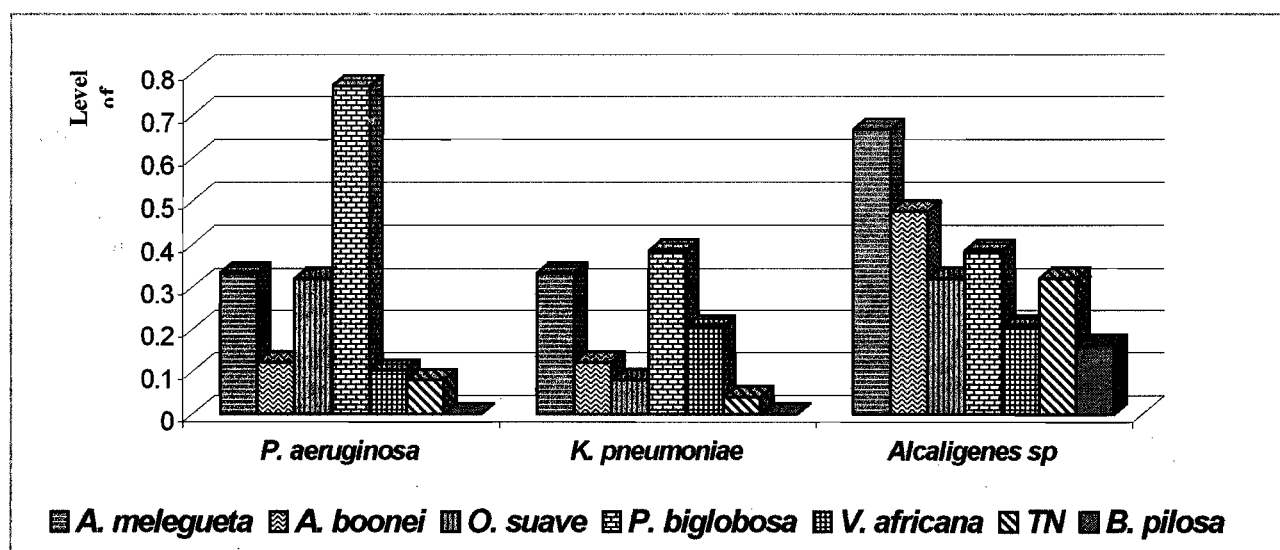


Fig. 2: Inhibitory activity of plant preparations on the test organisms

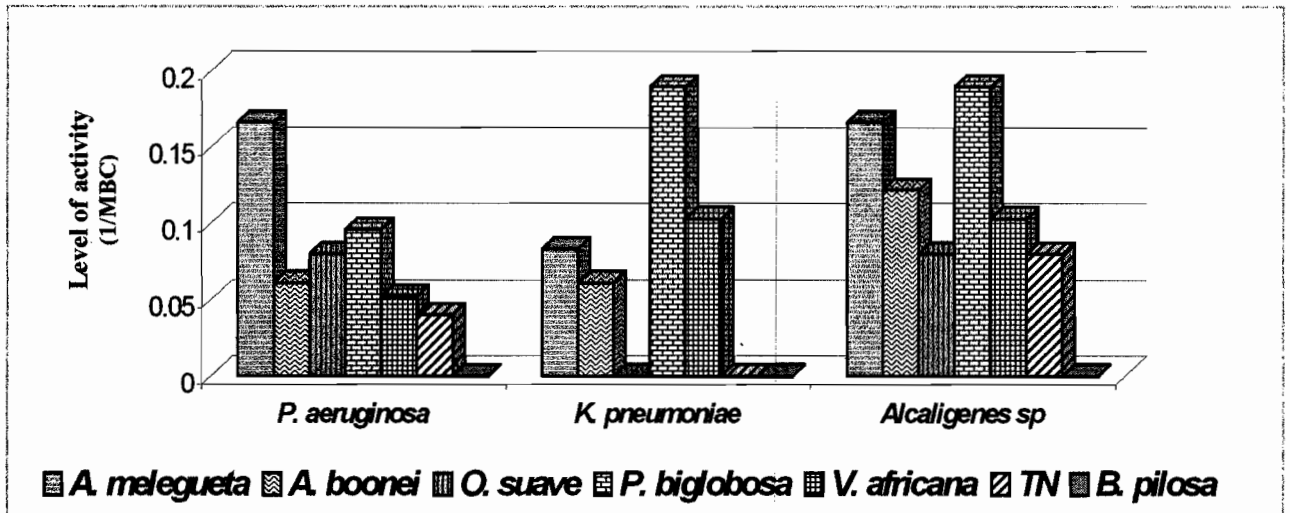


Fig. 3: Bactericidal activity of plant preparations on the test organisms

Significant bactericidal activity (shown by the 1/MBC values) was obtained with *Parkia biglobosa* and *Aframomum melegueta* extracts (Figure 3). The majority (67%) of MBC values obtained with these extracts was less than or equal to 6 mg/l (Table 3). The stem bark aqueous extracts from *Alstonia boonei* and *Voacanga africana* exhibited moderate bactericidal activities against all the test organisms. Generally, it was found that aqueous crude extracts were more active compared to methanol extracts and purified compounds

DISCUSSION

The epidemiological study showed that of the 77 isolates found, 44, 35 and 18% belonged to the *Pseudomonas*, *Proteus* and *Klebsiella* species, respectively. Because the *Alcaligenes* species was found only on two occasions (2%), it was not used in further testing and statistical evaluation. *Pseudomonas* species were not only the most prevalent pathogens, but also the most resistant against the commonly used commercialised antibiotics. This prevalence was very far above the usual levels obtained by Talarmin *et al.* (1996) who reported that *P. aeruginosa* is implicated in 8 to 10% of surgical wound infections. Because *P. aeruginosa* is known to grow in some antiseptic solutions (Govan, 1992) such as "betadine" which is widely used today in hospitals, it is likely that cross infection through wound dressing equipment and antiseptic solutions may have contributed significantly to the high occurrence of this pathogen. According to Govan (1992), patients infected with nosocomial *P. aeruginosa* should be

isolated from other patients, given that an outbreak may be difficult to control.

It is important to note that the relatively low prevalence (18%) of *Klebsiella* spp. as compared to the *Pseudomonas* and *Proteus* species (44% and 35%, respectively) should not veil the danger related to the presence of this pathogen. In fact, despite the low prevalence, *Klebsiella* spp. was found to exhibit a relatively high (60%) multi-drug resistance. The severity of some of the infections caused by multiresistant strains of this species, especially the respiratory and urinary tract infections, underlines the significance of these results. On the whole, more than 50% of the isolated *Pseudomonas* and *Klebsiella* species were resistant to 11 and 9 antibiotics, respectively. Although similar studies are not available from the literature to permit comparative analyses, these results by themselves indicate that the range of choice of drugs effective against these pathogens is very narrow. The true severity of the situation can be highlighted. The high cost of the effective antibiotics, when available, further complicates the situation. In general, although it is to be expected that nosocomial strains should exhibit multi-drug resistance (Tenover, 2001), the percentages of multiresistant isolates of *Pseudomonas* and *Klebsiella* species obtained in this study are very high since multi-resistance was considered as sensitivity to at most only one antibiotic out of the 15 tested. The percentage of multiresistant strains as well as the resistance percentage to the drugs of choice exhibited by the *Proteus* isolates remained for the most part within the expected range, i.e. lower than 40% (Jarvis, 1998), and may have posed comparatively less danger.

One of the striking findings of this study was the rapid rise in the occurrence of resistant strains to gentamicin among the isolates of *Pseudomonas*, *Proteus* and *Klebsiella* species (Figure 1) with the Spearman range test linear correlation coefficient (r) values of 0.800, 0.950 and 0.950, respectively. All the three pathogens showed progressively increasing resistance to gentamicin, which is the reference drug of choice for wound infections due to the above named pathogens. This may have been due to poor infection control through drug misuse, poor application of aseptic techniques or poor hospital hygiene. A socio-economic argument could also be advanced given that the epidemiological study period coincided with a point in time when hospital staff was claiming salary arrears of 12 to 18 months. This could have contributed to a general malaise and consequent lack of proper care by the health care workers.

The fact that most of medicinal plant preparations screened showed bactericidal and/or bacteriostatic activity against all the test pathogens lends support to their use in the traditional treatment and management of wounds and ulcers. The absence of activity of *B. pilosa* methanol extract on *P. aeruginosa* and *K. pneumoniae ssp*, as well as the total absence of activity of PAL (the compound purified from *E. chlorantha*), does not exclude the usefulness of these plants in the traditional treatment of wounds as reported in the literature (Tan *et al.*, 2000a; Tan and Nyasse, 2000). The active principles may have been missed in the process of extraction. The indiscriminate antimicrobial activities of the aqueous stem bark extracts of *P. biglobosa*, *A. boonei*, *A. melegueta* and *V. africana* can be attributed to their multiple bioactive constituents (Arvine, 1961; Connel, 1970; Menut *et al.*, 1991; Tan *et al.*, 2000c and Tringali *et al.*, 2000). However, further studies should be done to find out what are the actual active principles among these bioactive constituents. Some of the plant preparations (*B. pilosa*, *O. suave* and TN) were found to be only bacteriostatic. This suggests that the antibacterial constituents only stopped cell multiplication probably by rendering the culture medium non-conductive to cell growth without impairing the cell structure (Mims *et al.*, 1993). The germs were later able to grow when transferred to a drug free medium. Clinically, such bacteriostatic activity helps to stop the invading process of the

pathogen at the site of infection thus favouring rapid healing of the wound through leucocyte and connective tissue invasion and scar tissue formation. Given that the test organisms selected for the study were all multiresistant strains, the possibility of the use of medicinal plants as sources of effective drugs for the treatment of *P. aeruginosa*- and *K. pneumoniae*-infected wounds is an exciting and promising finding given the stubborn nature of these pathogens. Medicinal products prepared using crude plant extracts are becoming increasingly popular (Sofowora, 1993). Since *P. aeruginosa* and *K. pneumoniae* are responsible for other disease conditions in addition to wound infections, the antibacterial potential of the plant extracts studied here in disease conditions such as respiratory and urinary tract infections merits further investigation. The results demonstrate the need for wider studies to establish nation-wide nosocomial bacterial sensitivity patterns, as well as the search for alternative drugs of local plant origin against multiresistant nosocomial pathogens.

ACKNOWLEDGMENTS

We thank the Bafang Ad-Lucem Hospital, for the collaboration, financial and material support received during the first part of this work. We thank especially the Chief medical officer (Dr. N. JOUAKEP) and the Medical Officer in charge of the surgery ward (Dr. J. NGUEUMACHI) for their special assistance.

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Received: 18/11/2004

Accepted: 02/08/2005