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Full Length Research Paper

Incidence and susceptibility pattern of clinical isolates from pus producing infection to antibiotics and *Carica papaya* seed extract

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The prevalence of bacterial pathogens isolated from pus producing infections (wound, eye and ear) randomly collected from the Medical Microbiology Laboratory of University College Hospital (UCH),Ibadan, Nigeria was determined. Their susceptibility to selected antibiotics and *Carica papaya* seed extracts was investigated. Bacterial pathogen identity was confirmed based on standard methods which included, Gram stain reaction, colonial morphology on media, lactose fermentation, catalase,oxidase,coagulase and indole tests. The antibiogram was carried out using standard disc agar diffusion method employing commercially prepared antibiotic disc (Abtek Limited) of amoxycillin (25mcg), co-trimoxazole (25 mcg), nitrofurantoin (300 mcg), gentamicin (10 mcg), nalidixic acid (30 mcg),ofloxacin (30 mcg), augmentin (30 mcg) and tetracycline (10 mcg). A total of 58 isolates were obtained out of which 31 were from wound, 12 from ear and 15 from eye. 41 of the isolates were recovered from adults, while 17 were from children. *Staphylococcus aureus* was found to be the most common organism recovered (51.7%), followed by *Klebsiella* spp. (11%), *Pseudomonas aeruginosa* (11%),*Proteus* spp. (6.9%) and *Escherichia coli* (3.4%). From wound swabs, *S. aureus* isolates was the highest (51.6%) followed by *Klebsiella* spp. (22.6%), *Pseudomonas aeruginosa* and *Proteus* spp. (9.7% each) and *E. coli* (6.45%). In ear infection, *P. aeruginosa* accounted for 58.3%, *S. aureus* 25%, while *Klebsiella* spp. and Proteus spp. accounted for 16.6% each. In eye infection, *S. aureus and Klebsiella* spp. were more frequently isolated with 73.3 and 20%, respectively. The antibiogram studies showed that all the organisms were highly sensitive to ofloxacin as follows: *S. aureus* (80%), *Klebsiella* spp. (100%), *P. aeruginosa* (100%), *Proteus* spp. (100%) and *E. coli* (100%) while *P. aeruginosa* (63.6%), *S. aureus* (76.6%) and *E. coli* (100%) were sensitive to gentamicin and *Klebsiella* spp. (18.2%) and *Proteus* spp. (25%) were resistant. The organisms were resistant to all the other antibiotics tested namely: amoxycillin, co-trimoxazole, nitrofurantoin, nalidixic, augmentin and tetracycline. *C. papaya* extract showed antimicrobial activity which compared favourably with the commercial antibiotic discs against the Gram positive and negative bacteria tested, except *Klebsiella* spp. The highest activity was demonstrated against *P. aeruginosa.*

Key word: Susceptibility, bacterial pathogens, pus, infection, antibiotics, *Carica papaya* extract.

INTRODUCTION

Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths everyday (Ahmad and Beg, 2001). The most important reason for the use of antimicrobial agents is to cure or prevent infectious diseases by using the best available agent. The benefits to the individual who deserves treatment must be weighed against the risk of the emergence of resistant microorganisms to the public (Kunin, 1988).

Rapid development of multidrug resistance by micro-organisms to available antimicrobial agents has further complicated the threat of infectious diseases to human

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Table 1. Source and number of isolates used.

Sample	Children	Adult	Total
Wounds	4	27	31
Ear swabs	8	4	12
Eye swabs	5	10	15
Total	17	41	58

health. The choice of antimicrobial chemotherapy is initially dependent on clinical diagnosis. However, for many infections, establishing a clinical diagnosis implies determining possible microbiological causes which requires laboratory information from samples collected, preferably before antibiotic therapy is begun. Laboratory isolation and susceptibility testing of organisms make diagnosis to be established and also make drug selection more rational.

The local use of natural plants as primary health remedies due to their pharmacological properties is quite common in Asia, Latin America and Africa (Bibitha et al., 2002). Plants have the major advantage of still being the most effective and cheaper alternative sources of drugs (Pretorian and Watt, 2001). *Carica papaya* (family *Caricacea*) is a monosexual plant of Central American origin. The latex from the leaves has been used as antihelmintics for the treatment of infection of bacterial origin (Fajimi et al., 2001)

This research was undertaken to determine the prevalence of bacterial pathogens that are commonly responsible for pus producing infections of wound, eye and ear and to investigate the susceptibility of the organisms to selected antibiotics as well as seed extracts of *C. papaya*

MATERIALS AND METHODS

Collection and preparation of test clinical isolates

Fifty eight (58) bacterial isolates from wound, eye and ear clinical samples were randomly collected from the Microbiology Laboratory of University College Hospital (UCH), Ibadan, Nigeria. The isolates were obtained on nutrient agar slants and incubated at 37°C for 24 h. They were subcultured periodically.

The isolates were re-identified and confirmed using standard microbiological method which included, gram staining, colonial morphology on media, lactose fermentation, catalase, oxidase, coagulase and indole tests.

Antibiotic sensitivity testing

Antibiotic susceptibility testing was performed by the disc diffusion assay on Muller Hinton Agar as described by Bauer et al. (1966) using the following antibiotics disc: amoxycillin (25 mcg), cotrimoxazole (25 mcg), nitrofurantoin (300 mcg), gentamicin (10 mcg), nalidixic acid (30 mcg), ofloxacin (30 mcg), augmentin (30 mcg) and tetracycline (10 mcg). Interpretation of diameter of growth inhibition zone was done by using the manufacturer's manual. Organisms were scored as either sensitive or resistant.

Staphylococcus aureus ATCC 25923*, Pseudomonas aeruginosa* ATCC 27853 and *Esherichia coli* ATCC 25922 were used as control strains. Diameters of growth inhibition zones were recorded to the nearest mm for an average of 2 readings in each case.

Processing of plant materials

C. papaya fruits collected from Sagamu in Ogun state were authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The unripe pawpaw fruits were cut open and the seeds removed and put in clean polythene nylon. The seeds were air dried for two weeks to prevent loss of active compounds. They were grounded into powder with electric blender. 500 g of the powder was soaked in methanol (96%) for one week and then filtered. The filtrate was dried to yield a semisolid mass referred to as extract. The crude extract was reconstituted using 50% dimethylsuphoxide (DMSO) to obtain concentrations of 250, 200, 150, 100 and 50 mg/ml.

Determination of the activity of *C. papaya* seed extract on the bacterial pathogens

Antimicrobial assay

Antimicrobial activity of seed extract of the plant sample was evaluated by the cup plate agar diffusion method (Doughari et al., 2007) on Mueller Hinton agar. Aliquots of extract reconstituted in 50% dimethylsulphoxide (DMSO) and diluted to obtain concentrations of 250, 200, 150, 100 and 50 mg/ml were applied in each well in the culture plates previously seeded with the test organisms. A pre-diffusion time of 30 min was allowed at room temperature and the plates were incubated at 37°C for 24 h. *S. aureus* ATCC 25923*, P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as control strains. Diameters of growth inhibition zones were recorded to the nearest mm for an average of 2 readings in each case.

RESULTS

Out of the 58 clinical bacterial isolates used in this study, 31 (53.4%) were obtained from wound samples, 12 (20.6%) from ear samples and 15 (25.8%) from eye samples. 41 (70.6%) of all the isolates were recovered from adults, while 17 (29.3%) were from children (Table 1). The distribution of the different pathogens showed that *S. aureus* was the most prevalent (51.7%), followed by *Klebsiella* spp. (11%)*, Pseudomonas* (11%), *Proteus* spp. (6.8%) and *E. coli* (3.4%) (Table 2). From the wound samples, the distribution of the isolates recovered was as follows: *S. aureus* (51.6%), *Klebsiella* spp. and *Proteus* spp. (22.6%) each, *P. aeruginosa* (9.7%), *Proteus* spp. (9.7%) and *E. coli* (6.45%). *P. aeruginosa* was the most common organism isolated from ear specimen (58.3%) followed by *S. aureus* (25%), *Klebsiella* spp. (8.3%) and *Proteus* spp. (8.3%). *S. aureus* isolates was predominant in the eye samples (51.7%), followed by *Klebsiella* spp. (30%) and *P. aeruginosa* (6.7%).

The result of the antibiogram of the pathogenic isolates showed that all the organisms were highly susceptible to ofloxacin. The proportion of the isolates that were susceptible to ofloxacin were as follows: *S. aureus* (80%),

Table 2. Distribution of different pathogens.

Organism	Wound swab (%)	Ear swab (%)	Eye swab (%)	Total
S. aureus	16 (51.6)	3 (25)	11 (73.3)	30 (51.7)
Klebsiella spp.	7 (22.6)	1 (8.3)	3 (20)	11 (19)
P. aeruginosa	3 (9.7)	7 (58.3)	1 (6.7)	11 (19)
E. coli	2 (6.45)	0	0	2 (3.4)
Proteus spp.	3 (9.7)	1 (8.3)	0	4 (6.89)

Table 3. Antibiogram profile of bacterial isolates from pus producing infections.

Organism	Number	AMX no (%)	COT no (%)	NIT no (%)	GEN no (%)	NAL no (%)	OFL no (%)	AUG no (%)	TET no (%)
S. aureus	30	2 (6.67)	5 (16.7)	5 (16.7)	23 (76.6)	7 (23)	24 (80)	6 (20)	2 (6.67)
Klebsiella spp.	11	0	0	4 (36.3)	2 (18.2)	0	11 (100)	3 (27.2)	0
P. aeruginosa	11	0	9 (18.2)	0	7 (63.6)	2 (18.2)	11 (100)	0	0
Proteus spp.	4	0	0	0	1 (25)	0	4 (100)	0	0
E. coli	2	0	0	2 (100)	2 (100)	0	2 (100)	0	0

AMX, Amoxicillin; COT, co-trimoxazole; NIT, nitrofurantoin; GEN, gentamicin; NAL, nalidixic acid; OFL, ofloxacin; AUG, augmentin; TET, tetracycline; no = number.

Klebsiella spp. (100%), *P. aeruginosa* (100%), *Proteus* spp. (100%) and *E. coli* (100%) (Table 3).Only three of the organisms: *S. aureus* (76.6%), *P.aeruginosa* (63.6%) and *E. coli* (100%) were sensitive to gentamicin, while *Klebsiella* spp. (18.2%) and *Proteus* spp. (25%) were resistant to it. The pathogens were resistant to all the other six antibiotics tested. This showed that these categories of the isolates were multiresistant strains (defined as resistance to at least three classes of antibiotics). The comparative antibiotics susceptibility of bacterial isolates from the different sites of infection is shown in Table 4. *C. papaya* showed antibacterial activity against *P. aeruginosa*, while no activity was recorded against any of the *Klebsiella spp*. Isolates (Table 5).

DISCUSSION

The knowledge of the bacteriology of an infection and the laboratory susceptibility testing of micro-organism implicated could make drug selection in antimicrobial chemotherapy more rational. Suppurative infection of the skin, ear and eye are common occurrences in hospitalized and out patients. Wound infection is regarded as the most common nosocomial infection in surgical patients (Dionigi et al., 2001). It has been associated with increased trauma care, prolonged hospitals stay and treatment. (Bowler et al 2001).

The result of this study showed that *S. aureus* which accounted for 51.7% prevalence is the leading etiological agent of pus producing infections of the eye, ear and wound. *S. aureus* was the predominant isolates from wound (51.6%) and eye (51.7%) specimens. This is in agreement with the reports of Akonai et al. (1991), Shittu et al. (2003) and Bisi et al. (2005) that *S. aureus* is the leading etiological agent in wound infections. However, *P. aeruginosa* which accounted for only 11% of the total isolates from the three sites was the predominant organism of all the isolates obtained from ear specimen (58.3%). This is in agreement with earlier studies (Adedeji et al., 2007; Ogisi and Osamar, 1982; Selina, 2002) that rated *P. aeruginosa* as the most common bacteria isolated from mild to severe form of external otitis and chronic suppurative otitis media. Pus production is a common manifestation of infections due to *S. aureus* in tissues and sites with lowered host resistance such as damaged skin and mucous membrane,where it may produce skin lesion such as boil or surgical site infections. This possibly explain the high incidence of the organism from bacterial isolates obtained from this pus producing infection of eye, ear and wound in this and other similar studies.

The susceptibility studies showed that the bacterial pathogen was only highly susceptible to ofloxacin with varying degree of susceptibility to gentamicin. Resistance to commonly used antibiotics such as amoxycillin, cotrimoxazole, nitrofurantoin, nalidixic acid and tetracycline was predominant in the organisms.All the strains of the isolates used were resistant to the potentiated *B*-lactam antibiotic, augmentin. The phenomenon of multidrug resistance (defined as resistance to more than 3 classes of antibiotics) was noticed among the isolates in this study.

There was no defined pattern or difference in terms of sensitivity to antibiotic from the isolates obtained from different sites of infection of the eye, ear and wound. The emergence of resistance to antimicrobial agents is a

Table 4. Comparative analysis of susceptibility pattern of bacterial isolates from pus producing infection in relation to sample source.

Organism /source	Number	AMX no (%)	COT no (%)	NIT no (%)	GEN no (%)	NAL no (%)	OFL no (%)	AUG no (%)	TET no (%)
30 *S. aureus*(30)									
wound	16	2(12.5)	3(18.8)	4(25)	12(75)	2(12.5)	12(75)	3(18.8)	1(6.25)
Eye	11	0	1(9.1)	1(9.1)	8(72.7)	3(27.2)	9(81.8)	3(27.3)	1(9.1)
Ear	3	0	1(33.3)	0	3(100)	2(66.7)	3(100)	0	0
11 *Klebsiella* spp.									
wound	7	0	0	2(28.6)	0	0	7(100)	1(33.3)	0
Eye	3	0	0	1(33.3)	1(33.3)	0	3(100)	0	0
Ear	1	0	0	0	1 (100)	0	1(100)	0	0
11 *P. aeruginosa*									
wound	3	0	1(33.3)	0	0	1(33.3)	1(33.3)	0	0
Eye	1	0	1(100)	0	0	1(100)	1(100)	0	0
Ear	7	0	1(11.3)	0	5(71.4)	1(14.3)	7(100)	0	0
4 *Proteus* spp.									
wound	3	0	0	0	0	0	3(100)	0	0
Eye	0	-	-	-	-	-	-	-	-
Ear	1	0	0	0	1(100)	0	1(100)	0	0
2 *E. coli*									
wound	2	0	0	2(100)	2(100)	0	2(100)	0	0
Eye	0	-	-	-	-	-	-	-	-
Ear	0	-	-	-	-	-	-	-	-

AMX, Amoxicillin; COT, co-trimoxazole; NIT, nitrofurantoin; GEN, gentamicin; NAL, nalidixic acid; OFL, ofloxacin; AUG, augmentin; TET, tetracycline; no = number.

global public health problem, especially among pathogens causing nosocomial infection. (CDC, 1999). This is essentially due to improper use of antibiotics by health professionals, unskilled practitioners and laypersons, poor drug quality and inadequate surveillance programme (WHO, 1999).

Adequate epidemiological data on characterization and antibiotic susceptibility of bacterial pathogen is an essential ingredient to guide empiric antibiotic therapy in the ambulatory setting. This would reduce or eliminate errors in empirical selection of either ineffective or expensive drugs, prolonged hospitalization and higher mortality.

There is need for the evaluation of plants for antibacterial activity because of the twin effect of high cost and the development of resistance to synthetic/ conventional drugs used in orthodox practice.In this study, the seed extracts of *C. papaya* showed good antibacterial activity against the Gram negative than the Gram positive organisms tested. This result is at disparity with an earlier study that indicated that plant extracts are more active against Gram positive than Gram negative bacteria (Jigna et al., 2006). It however agreed with the findings of Doughari et al. (2007), where it was demonstrated that *C. papaya* extract showed a higher activity against Gram negative bacteria than Gram positive bacteria.

The demonstration of activity by the extract against the tested bacterial isolates provide the scientific basis for the local usage of the plant in the treatment of various pus producing infection such as wound infection and otitis media. It is also an indication that the plant is a potential source for the production of novel drugs for the treatment of pus producing infections.

This study showed that *S. aureus* is the leading aetiologic agent in pus producing infections and that ofloxacinand gentamicin may be used in the treatment of such infections before microbial and sensitivity test are carried out.

High level resistance to common antibiotics encountered in most of the isolates of bacterial pathogens is an indication that control measures have to be put in place, particularly in the administration of antibiotics in the hospitals. Patients should be educated on the consequences of indiscriminate use of antibiotics and there should also be an antibiotic resistance surveillance scheme.

The high sensitivity of the tested organism to ofloxacin may not serve any useful purpose in children where its use is contra-indicated. It should also be used with caution in adults because the emerging low level of resistance may become high in future due to selective

Table 5. Comparative antimicrobial activities of *C. papaya* seed extract with commercial antibiotics discs

Strain of organism	Inhibition zone (mm)				
	C. papaya seed extract (mg/ml)			GEN (mcg)	OFL(mcg)
	150	200	250	10	30
S1	8	12	12	9	27
S3	8	11	12	16	20
S5	10	10	12	17	23
S7	10	12	12	13	0
S9	8	10	11	19	26
S12	10	12	12	18	21
S13	8	10	11	12	22
S14	8	10	12	19	20
S15	8	10	12	14	19
S19	10	10	12	14	18
S20	8	10	12	21	20
S21	8	10	11	18	0
S30	10	10	11	19	23
Ps1	12	15	19	14	21
Ps3	12	15	18	0	28
Ps7	12	15	18	15	20
Ps8	10	12	17	15	23
Ps11	14	15	15	0	25
P1	12	16	18	13	25
P2	10	12	15	0	18
E1	10	10	12	14	21
E2	10	11	12	14	15

GEN = Gentamicin; OFL = Ofloxacin; S = *Staphylococcus aureus*; PS = *Pseudomonas aeruginosa*; P = *Proteus* spp; E = *Escherichia coli.*

pressure of exposure as a result of constant use because of arbitrary prescription of the antibiotic.

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