Studies On Susceptibility Of Methicillin –Resistent Staphylococcus aureus To Some Nigerian Honey

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Abstract: This study was aimed at determining the susceptibility of methicillin-resistant S. aureus (MRSA) isolates to some Nigerian honey. Sixty isolates of S. aureus were obtained from patients attending State Hospital, Jimeta Yola, Adamawa State. Twenty out of the sixty isolates were MRSA which were assessed for susceptibility or resistance to three (one processed and two crude) local honey samples in different concentrations and two commonly used antibiotics namely ciprofloxacin and ofloxacin using disk diffusion assay. All the twenty MRSA were susceptible to undiluted Sardauna plateau honey and its different concentrations of 50%, 25% and 13% (with growth inhibition zone ranging from 13 to 33 mm) but 25% of the isolates were resistant at concentrations of 6%. Against the MRSA isolates, undiluted Hong honey recorded 85% antibacterial activity, followed by 65%, 55%, and 5% respectively for its lower dilutions of 50%, 25% and 13% (with growth inhibition zone 12 or less than 12 mm). The undiluted Abuja honey sample recorded 85% antibacterial activity, followed by 35% and 15% respectively for its lower dilutions of 50% and 25%. Eighty five percent (85%) of the isolates were resistant to ofloxacin and 80% of the isolates were resistant to cipfrofloxacin (growth inhibition zone 20 mm or less for ciprofloxacin and 15 mm or less for ofloxacin, respectively). Values of the minimum inhibitory concentration and the minimum bactericidal concentration of S.P. honey were in the range of 0.4%-0.5% and 0.8 - 1% respectively whereas the values for H. honey and A. honey were in the range of 0.9-0.1% and 1.9-2.0% and 3.5-4.0% respectively. [Nature and Science 2010;8(2):98-108]. (ISSN: 1545-0740).

Keywords: MRSA, honey, methicillin, MIC, MBC

Introduction

MRSA, a major health problem worldwide, is a specific strain of the Staphylococcus aureus bacterium that has become resistant to all penicillin antibiotics such as methicillin and other narrow-spectrum beta-lactam penicillin antibiotics (Schito, 2006). This accounts for MRSA's serious and increasing threat to public health since Jevons first reported on the emergence of the strain in 1961 (Derek et al., 2005). Also, since 1996, vancomycin-resistant S. aureus (VRSA) has emerged against a drug considered the "last line of defense" when all other antibiotics have failed (Guignard et al., 2005). Wyllie et al (2006) reported a death rate of 34% within 30 days among patients infected with MRSA, and 27% death rate among patients infected with methicillin-susceptible Staphylococcus aureus (MSSA).

Studies have shown that MRSA is a growing health problem in many parts of the world, including Europe, America, Africa, the Middle East, and East Asia (Grundmann, 2006). Martha et al. (2009) isolated MRSA isolates from AIDS patients attending some public hospitals in Yola, Adamawa State, Nigeria. In the Netherlands, for instance, the annual number of MRSA strains submitted for epidemiological typing to the National Institute for Public Health and the Environment has risen from less than 200 in the early nineties to about 500 in 2001 (Simon et al., 2008).

A study was conducted on the prevalence and antibiotic susceptibility patterns of MRSA in eight large hospitals (>500 beds) in Africa and Malta from 1996 to 1997. Susceptibility to methicillin (oxacillin) and to other drugs was determined by E test (AB Biodisk, Solna, Sweden, 2001) on a total of 1440 clinical isolates of S. aureus. Methicillin resistance was detected in 213 (15%) of the 1440 isolates tested. The rate of MRSA was relatively higher in Nigeria, Kenya and Cameroon (21–30%), and below 10% in Tunisia, Malta, and Algeria. (Kesah et al, 2003). All MRSA isolates were sensitive to vancomycin, with MICs ≤ 4 mg/L. The isolates were also highly sensitive to ciprofloxacin, except in Kenya, Morocco, and Tunisia, where relative resistance to this drug was noted. Susceptibility to rifampin and fusidic acid seems to be correlated with the clinical use of these compounds. Only 46% of 59 MRSA strains analyzed were susceptible to rifampin, fusidic acid, and

ciprofloxacin. The majority (> 60%) of MRSA strains were multi resistant (Kesah et al, 2003).

Molan (1992) reported that honey has an inhibitory effect on about 60 species of bacteria including aerobes, anaerobes, gram-positives and gram-negatives. Antibiotic-resistant strains of Staphylococcus aureus have been studied and found to be as sensitive to honey as the antibiotic-sensitive strains of the same species. The MIC for 82 epidemic strains of methicillin-resistant Staphylococcus aureus (MRSA) was found to range from 3% to 8% (v/v) (Allen et al., 2000). Another study was conducted on 56 strains of vancomycin-resistant enterococci (VRE) and it has been shown that the MIC values were found to range from 5% to 10% (v/v) for manuka honey with activity due to a phytochemical component and a typical multifloral honey with activity due to hydrogen peroxide. Both the honey samples were collected from New Zealand. In another study, the MIC values for eight strains of MRSA isolated from swabs collected from acute and chronic wounds were all below 10% for honey used as antimicrobial agent (Molan, 1992).

Studies have shown that accurate detection of Oxacillin/Methicillin resistance can be difficult due to the presence of two subpopulations (one susceptible and the other resistant) that may coexist within a culture of Staphylococci (Brown et al.,2005). All cells in a culture may carry the genetic information for resistance, but only a small number may express the resistance in vitro. This phenomenon is termed heteroresistance and occurs in Staphylococci resistant to penicillinase-stable penicillins, such as oxacillin. Cells expressing heteroresistance grow more slowly than the oxacillin-susceptible population and may be missed at temperatures above 35 °C. That is why CLSI has recommended that test isolates against oxacillin, methicillin, or nafcillin be incubated at 33-35 °C (maximum of 35 °C) for a full 24 hours before reading (CLSI, 2005).

Given the threat posed by the growing problem of MRSA to public health, compounded by MRSA increased resistance to almost all new drugs, lack of currently available antimicrobials with rapid cidal activity against MRSA, and the global need for alternative effective anti-MRSA, the continued surveillance and control of MRSA infections, evaluation of honey from various flowery sources, geographic areas and processing against MRSA infection in humans is pertinent, indeed imperative (Kesah et al., 2003). The aim of this study was to compare/ascertain the extent of susceptibility of MRSA isolates to some locally processed or crude honey samples at their varied concentrations.

Materials and Methods

2.1 Collection of antibiotics and honey samples

Ciprofloxacin and Ofloxacin disks each of 5 µg/disk (Tyonex Nigeria Limited, Lagos, 2004) were obtained in a local laboratory consumable store (New Era Medical Diagnostic Laboratories) in Jimeta-Yola metropolis.

Three honey samples for this study were obtained from three local sources: processed honey from Betty Nnadi Farms Ltd, Abuja-FCT and crude honey from traditional bee farmers in Hong LGA, Adamawa State, and on the Mambila or Savannah plateau in Sardauna LGA, Taraba State, Nigeria. The honey samples were stored at room temperature in the dark until they were used for the experiments.

2.2 Collection of Specimens

A total of nine hundred samples were collected from eight pathological sources. Wound biopsy samples both of the needle aspiration and swab types and catheter urine specimens were collected by the clinicians in the ward and sent to the laboratory for isolation purpose. Patients not hospitalized were each given a sterile, wide-necked, leak-proof container for collection of urine samples. Specimens from Genito-Urinary tract were also collected using Swabs. Urethral discharge from male patients was collected using sterile cotton wool swab. Eye swabs were taken using swab sticks as described by WHO (2003) and cultured immediately to prevent enzymatic action killing any microbe present in the samples.

2.3 Isolation and Identification of Staphylococcus aureus

2.3.1 Isolation of S. aureus

To isolate pure colonies, the methods as described in CLSI (2005) were adopted. Two Mannitol Salt Agar (MSA) plates for each specimen were inoculated using the streak method.

2.3.2 Identification of S. aureus

The identification of the Staphylococci isolates was done following the procedures described by WHO (2003) as follows.

(a) Morphological and Cultural Characteristics

Incubated plates were examined for characteristic golden or white staphylococcal growth.

Isolates were examined microscopically using Gram staining procedure for Gram-positive cocci in clusters (GPCC), which suggest the presence of Staphylococcus species.

(b) Biochemical Characteristics

(i) Catalase activity— A wire loop was used to collect a speck of growth from each plate incubated for 24h (section 3.2.1) and emulsify in a drop of 3% hydrogen peroxide (H_2O_2) on

microscope slide. Formation of effervescence indicates presence of Staphylococci strains.

(ii) Coagulase test

Staphytect Plus test method:

Isolates were tested for coagulase production using Staphytect Plus test method. The reagents were purchased from Sanofi Diagnpostic Pasteur, France and the method was followed as instructed by manufacturer. One drop of test latex reagent was dispensed onto one of the circles on the reaction card and one drop of control latex was dispensed onto another circle.

A loop was used to pick up 5 average-size of suspected staphylococcal colonies onto a culture media plate and mix this in the control latex reagent. The colonies were smeared in order to cover the circle. A separate loop was then used to proceed in the same way with the test latex.

The card was rocked for 20 seconds and agglutination was observed under normal lighting conditions.

A result was reported positive if agglutination of the blue latex particles occurred within 20 sec. This identified the strain as a MRSA. A result was reported negative if no agglutination occurred and a smooth blue suspension remained after 20 sec in the test circle.

Coagulase activity using Pastorex Staph Plus®

Isolates were also tested for coagulase production using Pastorex Staph Plus® latex slide agglutination kits as instructed by the maker (Sanofi Diagnnostic Pasteur, France), to differentiate MRSA isolates from MSSA by detection of clumping factor (fibrinogen), Protein-A, and capsular polysaccharides found only in MRSA (Cruickshank et al., 2000).

Result was reported positive if agglutination of the blue latex particles occurred within 20 sec. This identifies strain as a MRSA. Result was reported negative if no agglutination occurred and a smooth blue suspension remained after 20 sec in the test circle. This identified the strain as a MSSA.

Twenty (20) MRSA and 40 MSSA isolates were identified and the MRSA were streaked on different mannitol salt agar slants. These were incubated for growth at 35 ^{O}C for 24h in an incubator and were stored at 4 ^{O}C in a refrigerator.

2.3.3 Antibacterial activity of cloxacilin against the MRSA isolates:

The antibacterial activity of cloxacilin at 5µg/ml against the MRSA isolates were determined using disk diffusion assay: Cloxacillin was selected because it is similar to methicillin in penicillinase resistance (Jawetz et al., 2000; Andrew, 2001). From each slant culture of MRSA isolates, five representative colonies were touched with a sterile loop and were suspended in sterile distilled water. Each MRSA water suspension was diluted in steps of 1:10 to adjust the MRSA suspension to 1x 10^{8} density equal to the 0.5 McFarland standards before inoculation (NCCLS, 1997) and were used for subsequent antimicrobial susceptibility test.

A loopful of culture of each of MRSA grown in MSA at 35 ^{0}C for 24h was surface spread evenly on 4% NaCl supplemented Mueller-Hinton agar (MHA) in Petri dishes. Cloxacilin disks were each picked with a pair of sterile forceps and applied to each uniformly seeded area of the plate, spaced out so that their centers were at least 2 cm apart, incubated aerobically at 35 ^{0}C for 24h.

2.4 Disk diffusion assay to determine zones of growth of inhibition of honey and ciprofloxacin and ofloxacin

The honey samples collected were treated to 40 ^{0}C in a water-bath and various dilutions 50%, 25%, 13%, 6% (v/v) honey were prepared using sterile distilled water. These solutions were used to saturate paper disks for assays to determine zones of inhibition against MRSA growth.

The antibacterial activity of different honey samples and the two selected antibiotics were determined using the procedure as described in section 2.3. The concentrations of the antibiotics used in this study were Ciprofloxacin 5µg/ml and Oflaxacin 5µg/ml. The plates were then incubated at 35 ^{0}C for 24h. For honey samples, a zone diameter that was 13 mm or above was reported sensitive and a zone of diameter of 12 mm or less was reported as resistant. The sensitivity/resistance profile of honey was determined using cloxacillin at 5 µg/ml concentration. For ciprofloxacin and ofloxacin, a zone diameter that was 21 mm or above was reported sensitive and a zone of diameter of 20 mm or less was reported as resistant and for ofloxacin, a zone diameter that was 16 mm or above was reported sensitive and a zone of diameter of 15 mm or less was reported as resistant (WHONET, 2006).

2.5 Determination of MIC of Different Honey Samples

This was done following the procedure as described in NCCLS (1984). Doubled strength (5.0 ml) of MHB were dispensed separately in six test tubes of 10 ml capacity into which the following were added: Graded volumes (0.5ml—4ml) of honey samples, (4.8 ml—0.8 ml) of sterile, distilled water and (0.2 ml) of 24h pure culture of MRSA broth. The MRSA broth was prepared as described in 2.4.1. The test tube which served as a control contained no honey but MHB, distilled water, and the 24h incubated pure culture of MRSA broth. All the test tubes were then incubated at 35 ^{0}C for 24h in a B28 liter Incubator (WTB Binder

Labortechnik GmbH, Germany) and observed visually for growth. The highest dilution of the antimicrobial that showed no visible growth of the organism was taken as the minimum inhibitory concentration.

The same procedure was applied for the other honey samples and the two standard antibiotics.

2.6 Determination of minimal bactericidal concentration (MBC) of honey

A loopful of broth was collected from each of the tubes in the MIC test that showed no visible growth and streaked on Mueller-Hinton agar plates and incubated at 35 ^{0}C for 24h in a B28 liter Incubator and observed for bacterial growth. The MBC was determined by the highest dilution at which there was no visible growth on the solid media.

2.7 Data Analysis

WHONET 5.4, World Health Organization (WHO, 2006) Data-base Software for managing laboratory test results, was used for data analysis to achieve the objective(s) of this study.

Results

3.1 Isolation and identification of S. aureus

Out of nine hundred samples examined, sixty pure cultures of Staphylococcus aureus isolates were obtained from eight pathological sites of patents in Specialist Hospital, Jimeta-Yola, Adamawa State, Nigeria.

S. aureus isolates collected from each of wound swab, urine specimens , ear swab and urethral swab was 17% followed by eye swab (13%), high vaginal swab (13%), wound biopsy (3%) and catheter urine (3%). Table 1 shows the distribution of S. aureus isolates according to their pathological sites.

3.2 Isolation and identification of MRSA

Of the sixty isolates of S. aureus, twenty were identified to be methicillin-resistant S. aureus (MRSA) strains from five pathological sites. Forty percent of MRSA isolates were collected from wound swab followed by eye swab (20%). HVS (20%), UTI(15%) and catheter urine (5%). Table 1 shows the distribution of MRSA isolates according to their pathological sites. The result shows that, out of the sixty isolates of S. aureus, (20) 33.3% were MRSA strains. The diameter of zones for all the MRSA isolates against methicilin were in the range of 6-12 mm.

3.3 Susceptibility Testing

3.3.1 Antimicrobial activity of S.P.Honey and two standard antibiotics against MRSA isolates

The MRSA isolates from eye swabs were highly susceptible to both diluted and undiluted honey from Sadauna Plateau and this was followed by isolates from HVS, wound infection, catheter urine and lastly the wound infection (Table 2).

Undiluted Sardauna Plateau honey sample and its different concentrations (50%, 25%, and 13%) recorded 100% antibacterial activity, followed by 75% for 6% dilution against the MRSA isolates. 85% of the isolates were resistant to ofloxacin and 80% of the isolates were resistant to cipfrfloxacin.

Table 5 shows the percentage resistance profile of MRSA to S.P. Honey and two standard antibiotics.

3.3.2 Antimicrobial activity of Hong Honey and two standard antibiotics against MRSA isolates

Against the MRSA isolates, undiluted Hong honey recorded 85% antibacterial activity, followed by 60%, 55%, and 5% respectively for its lower dilutions of 50%, 25% and 13%. The results are shown in Table 3. Table 5 shows the percentage resistance profile of MRSA to Hong honey and two standard antibiotics.

3.3.3 Antimicrobial activity of Abuja Honey and two standard antibiotics

The undiluted Abuja honey sample recorded 85% antibacterial activity, followed by 35% and 15% respectively for its lower dilutions of 50% and 25%. Table 4 shows the sensitivity of the isolates to the Abuja honey sample and two standard antibiotics, while Table 5 shows the resistance profile of MRSA to Abuja honey and two standard antibiotics.

3.4 MIC and MBC of antimicrobials and their effects on MRSA isolates

Values of the minimum inhibitory concentration and the minimum bactericidal concentration of Sarduna Plateau honey were in the range of 0.4%-0.5% and 0.8 - 1% respectively whereas the values for Hong honey and Abuja honey were in the range of 0.9-0.1% and 1.9-2.0% and 3.5-4.0% respectively. The values of MIC and MBC for ciprofloxacin and ofloxacin against the MRSA isolates were in the range of 0.5-1.0 % respectively.

Table 1: Distribution of S. aureus and MRSA isolates according to their pathological sites

Pathological source	No. of S. aureus	% of S. aureus	No. of MRSA	% of MRSA
Eye swab	8	13.00	4	20.00
Wound swab	10	17.00	8	40.00
Urinary tract infection	10	17.00	3	15.00
Catheter urine	2	3.00	1	5.00
Ear swab	10	17.00	-	-
Urethral swab	10	17.00	-	-
High vaginal swab	8	13.00	4	20.00
Total	60	100.00	20	100.00

Table 2: Growth Inhibition Zone Size (in mm) of S. P. Honey, Ciprofloxacin and Ofloxacin against MRSA isolates

Isolate No.	Specimen site	Growth Inhibition Zone Size (mm) Sardauna Plateau Honey (%) (x_1)	(x_2)	(x_3)	(x_4)	(x_5)	CIP	OFX
1	ey	33	30	27	23	17	21	15
2	ey	30	25	22	15	13	9	15
3	wd	30	25	22	15	13	16	10
4	ey	25	20	17	13	13	16	12
5	ey	32	30	27	22	11	21	12
6	HVS	28	24	23	18	11	21	6
7	wd	26	24	21	16	12	20	6
8	HVS	24	22	19	14	10	9	16
9	HVS	28	24	21	16	10	6	16
10	HVS	25	20	17	13	11	6	6
11	UTI	24	20	17	13	11	10	12
12	wd	24	20	17	13	9	6	6
13	cur	23	19	16	13	10	12	12
14	wd	26	24	21	16	12	15	6
15	UTI	25	20	17	15	10	15	12
16	UTI	24	20	17	16	12	18	8

17	wd	22	19	16	13	11	12	6
18	wd	21	20	17	15	14	22	6
19	wd	26	24	21	17	10	12	10
20	wd	20	18	15	13	12	11	11

Ey (eye swab)
HVS (high vaginal swab)
Cur (catheter urine)
UTI (urinary tract infection)
Wd (wound swab)
(x_1) undiluted Honey
X_2 (50% Honey)
X_3 (25% Honey)
X_4 (13% Honey)
X_5 (6% Honey)
CIP (Ciprofloxacin 5 µg)
OFX (Ofloxacin 5 µg)

Table 3: Diameter of zones (mm) of Hong Honey and two standard antibiotics against MRSA isolates

Isolate No.	Specimen site	Growth Inhibition Zone Size (mm) Hong Honey (%) (x_1)	(x_2)	(x_3)	(x_4)	(x_5)	CIP	OFX
1	ey	14	13	13	13	8	21	15
2	ey	17	13	13	11	10	9	15
3	wd	13	14	13	11	9	16	10
4	ey	24	20	18	12	8	16	12
5	ey	32	20	19	10	8	21	12
6	HVS	16	13	14	8	6	21	6
7	wd	23	20	15	10	10	20	6
8	HVS	16	15	14	6	7	9	16
9	HVS	13	14	13	8	8	6	16
10	HVS	31	14	13	10	8	6	6
11	UTI	24	16	14	9	7	10	12
12	wd	15	13	12	9	9	6	6
13	cur	18	12	11	10	10	12	12
14	wd	23	12	10	10	9	5	6
15	UTI	20	10	12	10	10	15	12
16	UTI	18	12	11	12	10	18	8
17	wd	19	12	11	10	10	18	9
18	wd	10	8	8	6	6	12	6
19	wd	12	10	9	6	6	12	10
20	wd	12	12	10	10	8	11	11

Key:
Ey (eye swab)
HVS (high vaginal swab)
Cur (catheter urine)
UTI (urinary tract infection)
Wd (wound swab)

(x_1) undiluted Honey
X_2 (50% Honey)
X_3 (25% Honey)
X_4 (13% Honey)
X_5 (6% Honey)
CIP (Ciprofloxacin 5 µg)
OFX (Ofloxacin 5 µg)

Table 4: Growth Inhibition Zone Size (mm) of Abuja Honey and two standard antibiotics against MRSA isolates

Isolate No.	Specimen site	Growth Inhibition Zone Size (mm) Abuja Honey (%) (x_1)	(x_2)	(x_3)	(x_4)	(x_5)	CIP	OFX
1	Ey	15	14	13	11	6	21	15
2	Ey	14	14	13	6	6	9	15
3	wd	15	14	13	11	6	16	10
4	Ey	22	18	12	10	10	16	12
5	Ey	30	20	10	9	8	21	12
6	HVS	14	13	10	9	8	21	6
7	wd	20	15	10	9	10	20	6
8	HVS	13	11	10	8	6	9	16
9	HVS	14	10	8	6	6	6	16
10	HVS	18	10	10	8	8	6	6
11	UTI	14	12	10	8	6	10	12
12	wd	19	12	10	8	6	6	6
13	Cur	18	12	11	8	6	12	12
14	wd	15	10	10	8	6	15	6
15	UTI	14	12	10	8	7	15	12
16	UTI	14	11	6	6	6	18	8
17	wd	14	12	10	8	6	18	9
18	wd	10	10	8	6	6	12	6
19	wd	12	10	8	6	6	12	10
20	wd	11	10	8	6	6	11	11

Key: Ey (eye swab)
HVS (high vaginal swab)
Cur (catheter urine)
UTI (urinary tract infection)
Wd (wound swab)
(x_1) undiluted Honey
X_2 (50% Honey)
X_3 (25% Honey)
X_4 (13% Honey)

X_5 (6% Honey)
CIP (Ciprofloxacin 5 µg)
OFX (Ofloxacin 5 µg),,

Table 5: Percentage resistance profile of MRSA to S.P. Honey, Hong honey, Abuja honey and the two standard antibiotics

Resistance profile	% of Isolate		
	S.P	H	A
OFX	10	-	-
CIP OFX	15	-	-
X_5 OFX	15	-	-
X_5 CIP	10	-	-
X_5 CIP OFX	50	10	-
X_4 X_5 OFX	-	5	-
X_4 X_5 CIP	-	10	5
X_4 X_5 CIP OFX	-	30	10
X_3 X_4 X_5 CIP OFX	-	10	10
X_2 X_3 X_4 X_5 CIP	-	5	-
X_2 X_3 X_4 X_5 CIP OFX	-	15	50
X_1 X_2 X_3 X_4 X_5 CIP OFX	-	15	15
X_3 X_4 X_5 OFX	-	-	5
X_3 X_4 X_5 CIP	-	-	5
Total	100	100	100

Key:
X_1 (undiluted Honey)
X_2 (50% Honey)
X_3 (25% Honey)
X_4 (13% Honey)
X_5 (6% Honey)
CIP (Ciprofloxacin 5 µg)
OFX (Ofloxacin 5 µg)

Discussion

The 33.3% of MRSA strains, out of the sixty isolates of S. aureus, isolated from patients attending Specialist Hospital, Jimeta, Yola indicate that there must have been a high prevalence of MRSA infections among patients, who came from Jimeta-Yola and its environs to attend the hospital (Table 1). Kesah et al, 2003 conducted a study on prevalence of MRSA in

large eight hospitals in Africa and Malta from 1996 to 1997 and observed that the rate of MRSA was relatively high in Nigeria, Kenya and Cameroon. These patients may be responsible for hospital acquired and community acquired infection. Hanselmann et al., 2006 reported that recently there was a shift in the epidemiology of MRSA infections to community-associated infections in case of significant illness and death. In view of the search for a solution to the problem of antibiotic resistant strains, this study highlights the potential of honey as a suitable and affordable first-line therapeutic agent against pathogens, particularly MRSA.

Variability in antimicrobial activity of honey samples from different floral and geographic sources against several strains of MRSA known to cause human diseases has been described (Willix et al., 1992). Its antimicrobial activity varies with origin, geographic area and processing against some pathogens, including methicillin-resistant Staphylococcus aureus (MRSA) (Molan, 2001; EARSS, 2004). In this study, all MRSAs tested showed different susceptibilities to each of the test honey samples. The inhibitory action of different honey samples was compared with that of two commonly used antibiotics namely ciprofloxacin and ofloxacin each at 5 µg/ml concentration. Seventy percent of the MRSA isolates tested showed susceptibility to S.P. honey sample at a low concentration of 6% signifying that more than one antimicrobial factor are present in honey (Table 2). Weston et al. 2000 reported that in addition to hydrogen peroxide, non-peroxidase components such as lysozyme, phenolic acids , flavonoids present in honey also contribute to the antibacterial activity of honey. The concentrations of these components differ in different types of honey samples. Carotenoids are also reported to be present in some honey especially the darker honey (Frankel et al, 1998). Some componens mainly 3,4,5-trimethoxy benzoate, methyl-4-hydroxy-3,5-dimethoxy benzoate and 3,4,5 trimethoxy benzoic acid responsible for the exceptionally high antibacterial activity of manuka honey were isolated by testing fractions of the honey for activity against S. aureus (Russel, 1983).

The anti-MRSA activity recorded in this study for honey was consistent with earlier reports on its curative and antibacterial properties (Willix et al., 1999). MRSA showed a susceptible level of 100% to the undiluted honey sample from Sardauna Plateau, followed by 75% susceptible level at 6% concentration. In this study, zones of growth inhibition of 13 mm or more for undiluted honey and 50% honey dilutions in disk diffusion proof the basis for offering some honey from some flora as a unique treatment in MRSA infections (Allen et al., 2000; Derma Sciences, 2008).

MRSA showed the highest susceptible level of 100% to the undiluted S.P. honey sample followed by 85% susceptible level at 6% concentration and is suggested as the treatment of choice against the pathogens, considering the huge impact of the strains as agents of nosocomial infections worldwide The present study has shown that compared with the honey samples and their 50% to 6% concentrations, ciprofloxacin and ofloxacin used in concentrations of 5µg/ml were less active on the MRSA isolates. The resistance levels among the MRSA isolates were 85% and 80% for ciprofloxacin and ofloxacin respectively, compared with 25% resistance levels at 6% concentration of honey from Sardauna Plateau and 15% and 16% resistance levels for undiluted Hong and Abuja honey samples respectively. These results find an analogy in an earlier report (Molan, 2000) of more effective treatment of bacterial infected burn wounds with honey than with silver sulphadiazine, a recognized antibacterial ointment.

The values of MIC ands MBC of the antimicrobials showed that their initial inhibition of the MRSA isolates did not stop only at prevention of growth (bateriostasis), but also extended to killing of the bacteria (bactericidal) activity. Three of the MRSA isolates showed total resistance both to Hong and Abuja honey samples, and to the two standard antibiotics. The isolates, however, were susceptible to S. P. honey.

Based on the results obtained in this study, it can be concluded that some honey from some flora has the potential as a suitable and affordable first-line therapeutic agent against pathogens, particularly MRSA. It may, however, be recommended that further understanding of the active species in the potency of honey is needed to optimize their selection and use as first- or -second line antimicrobials in various diseases caused by MRSA.

REFERENCES

Andrew, J. M. (2001). BSAC Working Party Report on Susceptibility Testing.
BSAC standardized disk susceptibility testing method. Journal of Antimicrobial Chemotherapy 48 (1): 43—57.

Allen, K. L., Hutchinson, G., and Molan, P.C. (2000).The potential for using
honey to treat wounds infected with MRSA and VRE: Text of a paper presented at the First World Wound Healing Congress, 10-13th September 2000, Melbourne, Australia. Medicine Digest. XXI, supplement 4, 61– 65.

Brown, D. F. (2005). Detection of methicillin/oxacillin resistance in staphylococci.
Journal of Antimicrobial Chemotherapy 48(1): 65–70.

CLSI (Clinical and Laboratory Standards Institute). (2005). Performance standards
for antimicrobial susceptibility testing. CLSI approved standard M100-S15.
Clinical and Laboratory Standards Institute, Wayne, PA.

Cruickshank, R., Duguid, J.P., Mornion, D.P.M., Swain, R.H.A. (2000). Medical
Microbiology (20th edition). Edinburgh: Churchill Livingston.

Derek, F. J., David, I. E., Peter, M. H., Donald, M., Geoffrey, L. R., Kevin, J. T.,
Michael, W. D. W. (2005). Guidelines for the laboratory diagnosis and
susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA). Journal of Antimicrobial Chemotherapy 56 (6): 1000-1018.

Derma Sciences. (2008). Randomized Controlled Clinical Trial Shows Derma
Sciences MEDIHONEY(TM) Eradicates MRSA From Chronic Venous Ulcers. Journal of Wound Care 16: 325–328.

Diekema, D. J., Pfaller, M. A., Turnidge, J., Verhoef, J., Bell, J., Fluit, A. C.,
Doern, G. V. & Jones, R. N. (2000). Genetic relatedness of multidrug-
resistant, methicillin (oxacillin)-resistant Staphylococcus aureus
bloodstream isolates from SENTRY Antimicrobial Resistance Surveillance
Centers worldwide, 1998. Microbial Drug Resistance 6: 213-221.

EARSS (European Antimicrobial Resistance Surveillance System) (2004). Annual
Report EARSS-2003. RIVM, Bilthoven, The Netherlands.

Frankel, S. , Robinson, G.E. and Berenbaum, M.R. (1998) Antioxidant capacity and correlated
Characteristics of fourteen unifloral honeys J. Apic. Res. 37(1): 27-31

Grundmann, H. (2006). MRSA: A Growing Global Health Problem. European
Antimircrobial Resistance Surveillance System (EARSS), Center for Infectious Disease Epidemiology, National Institute for Public Health, Bilthoven, Netherlands.

Guignard, B., Entenza, J.M., and Moreillon, P. (2005). "Beta-lactams against
methicillin-resistant Staphylococcus aureus". Current Opinion in
Pharmacoloy 5 (5): 479-489.

Hanselman, B.A., Kruth, S.A., Low, D.E., Wiley, B.M., McGeer, A. and Weese, J.S. (2006)
Methicillin-resistant Staphylococcus aureus colonization in veterinary personnel J.
Infect. Dis.12:1933-1937

Kesah, C., Redjeb, S.B., Odugbemi, T. O., Boye, C. S. -B. Dosso, M., Achola, J.
O. N., Koulla-Shiro, S. Benbachir , M., Rahal. K., and Borg, M. (2003).
Prevalence of methicillin-resistant Staphylococcus aureus in eight African
hospitals and Malta. Clinical Microbiology and Infection. 9 (2): 153—156.

Abraham, M., A. , De, N., Sudi, I.Y. and Mayori, L. (2009) Isolation of Methicillin-resistanr Staphylococcus aureus (MRSA) from AIDS Patients Attending State Specialist Hospital, Yola and Federal Medical Centre, Yola, Adamawa State, Nigeria Report and Opinion 1(6): 103-107

Mir, N., Sanchez, M and Baquero, F. (1998). Soft salt-mannitol agar-cloxacillin
test: a highly specific bedside screening test for detection of colonization
with methicillin-resistant Staphylococcus aureus. Journal of Clinical
Microbiology 36: 986–989.

Molan, P. C. (1992). The antibacterial activity of honey: 1. The nature of the
antibacterial activity. Bee World 73: 5-28.

NCCLS (National Committee for Clinical Laboratory Standards). (1984).
Performance standards for antimicrobial disc susceptibility tests. M2—A3. NCCLS. Villanova, PA, USA.

Russel, K.M.(1983) The antibacterial properties of honey Journal of Agricultural and Food
Chemistry 38(1):150

Schito, G.C. (2006). The importance of the development of antibiotic resistance in Staphylococcus aureus. Clinical Microbiology and Infections 12(1): 3-8.

Simon, A., Traynor, K., Santos, K., Blaser, G., Bode, U., and Molan, P. (2008). Medical Honey for Wound Care—Still the 'Latest Resort'? Annals of Oncology, 175.

Taormina, P.J., Nemira,B.A., Beuchat, L. R.(2001). Inhibitory activity of honey against food-born pathogens as influenced by the action of hydrogen peroxide and level of antioxidant power. International Journal of Food Microbiology 59: 217—225.

Weston, R.J., Brocklobank, L.K., Lu, Y. (2000). Identification and quantitative levels of antibacterial components of some New Zealand honeys. Food Chemistry 70: 427—435.

Willix, D. J., Molan, P. C., Harfoot, C. G. (1992). A comparison of the sensitivity of wound infecting species of bacteria to the antimicrobial activity of manuka honey and other honey. Journal of Applied Bacteriology 73: 388-394.

WHO. (World Health Organization). (2003). Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public importance in the developing world. Geneva. Pp. 103- 122.

WHO (World Health Organization). (2006). WHONET 5.4 Update notes. Geneva.

Wyllie, D.H., Crook, D.W., Peto, T. (2006). Mortality after Staphylococcus aureus bacteremia in two acute hospitals in Oxfordshire, 1997—2003: cohort study. British. Medical Journal 333: 281—284.

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