IN VITRO ANTI-STAPHYLOCOCCAL ACTIVITY OF HONEY AND TWO STANDARD ANTIBIOTICS (CLOXACILLIN AND AMPICILLIN)

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SUMMARY. Fifty bacterial isolates comprising *Staphylococcus aureus* and *Staphylococcus albus* from six pathological sources were exposed to honey and its fractional dilutions as also as to two standard antibiotics - cloxacillin (5 mg/ml) and ampicillin (10 mg/ml) - in an agar-cup diffusion sensitivity test. The undiluted honey showed antibacterial activity against almost 100% of the bacterial isolates while its 1:2 to 1:10 fractional dilutions also had antibacterial property against the isolates in decreasing order of activity. Cloxacillin recorded higher antibacterial activity than ampicillin but both exerted less antibacterial activity than honey. The therapeutic application of honey, following clinical trials, should be of relevance in treating bacterial infections and, in particular, those due to the two *Staphylococcus* species tested.

Introduction

Staphylococcus aureus and *Staphylococcus albus* have both been recognized as part of the normal microbial flora and persistent pathogens in man.[1,2] More specifically, varieties of superficial and suppurative infections have been associated with *S. aureus*, while bacteraemia has been linked to *S. albus* following infections of cannulae, indwelling catheters, shunts, and other appliances positioned in the body.[3] Pathogenic strains of *S. albus* have been reported[4] to owe their virulence to the production of slime, which forms a biofilm on the surface of prosthetic devices, while *S. aureus* produces a number of enzymes and toxins, notable among which are coagulase, deoxyribonuclease, and enterotoxin B, which accounts for food-poisoning.

R plasmid genes mediating production of antibiotic-inactivating enzymes have been largely implicated in the resistance of *S. aureus* to antibiotics, particularly the β-lactams, including cloxacillin, cefuroxime, cefotaxime, methicillin, and vancomycin.[4-6] *S. albus* has a record of 75% of its strains having resistance to naficillin and it can also sequester itself from the circulation and antimicrobial drugs when it occurs in prosthetic devices.[2,7]

Honey is widely regarded as a food supplement owing to its greater rate of absorption than that of table sugar, its nutritive properties, and its easy digestibility.[8-11] It is also a recognized income-earner.[12] To this regard, harvesters, collectors, and hawkers of honey have significantly increased in our country, Nigeria.

Interestingly, honey has been associated with curative and antimicrobial effects. Heart and liver diseases, coughs, constipation, gastrointestinal disturbances, measles, ulcers, and wounds have all been treated with honey.[13] Higher antibacterial activity against infections in burn wounds has been reported for honey than for the antibacterial ointment silver sulphadiazine.[14] Laboratory isolates of *S. aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, as also diarrhoeal bacterial isolates, had their respective growth inhibited by honey.[15,16]

In environments where sanitation remains at a low ebb, *S. aureus* and *S. albus* are important environmental pollutants, especially in tropical developing countries. Hence the need for a readily available and affordable treatment for infections due to these organisms, in view of their resistance to the cheaper β-lactam antibiotics. This study reports on the comparative activities of honey and two standard antibiotics (ampicillin and cloxacillin) against two staphylococcal species from six pathological sources.

Materials and methods

Bacterial isolates

Pure cultures of bacterial isolates from different pathological sources (*Table I*) were obtained from the Routine

Table I - The bacterial isolates and their pathological sources

Pathological source	*S. aureus*	*S. albus*
Eye swab	8	10
Wound swab	13	3
Urine	3	-
Wound biopsy	1	-
Aspirate	-	10
Ear swab	-	10
Unidentified source	5	5
	30	20

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Honey

Honey was obtained from a pure natural honey collection centre in Ibadan, South West Nigeria. It was used undiluted and also as fresh fractional dilutions in sterile distilled water (1:2, 1:4, 1:6, 1:8, and 1:10).

Ampicillin and cloxacillin (as sodium salt BP)

The two antibiotics were obtained in a local pharmacy store in Ibadan in ampoule vials as powder for injection. Cloxacillin was used at 5 μg/ml and ampicillin at 10 μg/ml in aqueous dilution, reflecting hospital practice in the use of antibiotic discs.

Sensitivity test

An overnight nutrient broth (OXOID) culture of each bacterial isolate, equivalent to 10^7 cells/ml, was diluted 1:100 in sterile distilled water. A seeded pour-plate was prepared from a mixture of 0.1 ml from the 1:100 dilution and 20 ml molten nutrient agar. Wells were dug with a flame-sterilized size 3 cork-borer of 6.5 mm internal diameter. The undiluted honey and its respective fractional dilutions, as well as the 5 μg/ml and 10 μg/ml of cloxacillin and ampicillin, were filled in turn into the wells. The culture plates were allowed to remain on the bench for a pre-incubation diffusion period of 2 h, followed by incubation in an upright position for 24 h at 37 °C. The bacterial isolates were then assessed for sensitivity or resistance, using the zone of growth inhibition as an index.[18]

Results

The sensitivity test revealed for *S. aureus* and *S. albus* a better susceptibility pattern to undiluted honey and its lower dilutions (1:2 and 1:4) than to cloxacillin and ampicillin. This was evident in the zones of growth inhibition produced by undiluted honey, which varied from 6 to 33 mm against the two *Staphylococcus* spp. isolates. Cloxacillin produced zones of growth inhibition varying from 6 to 24 mm against *S. aureus* and 9.5 to 18 mm against *S. albus*. With ampicillin, the range was 8 to 28 mm against *S. aureus* and 10 to 21 mm against *S. albus* (*Table II*). Undiluted honey thus recorded 96.6% antibac-

Table II - Some results of sensitivity tests on honey and two standard antibiotics against the clinical isolates of *S. aureus* and *S. albus*

Isolate	Clinical source	Honey - zone of growth inhibition (mm)					Antibiotics	
		0*	1:2	1:4	1:6	1:8	Cloxacillin (5 μg/ml)	Ampicillin (10 μg/ml)
S. aureus	Eye swab	13	9	9	–	–	19	–
S. aureus	Eye swab	16	10	10	–	–	8.5	–
S. aureus	Eye swab	10.5	10	10	–	–	–	10
S. aureus	Eye swab	23	19	19	–	–	–	20
S. aureus	Eye swab	31	19	18	10	6	21	15
S. aureus	Wound swab	15	7	5	–	–	16	–
S. aureus	Wound swab	–	–	–	–	–	20	–
S. aureus	Wound swab	23	21	9	–	–	–	–
S. aureus	Wound biopsy	15	15	15	7	–	12	21
S. aureus	Urine	32	13	13	–	–	20	–
S. albus	Eye swab	23	15	15	–	–	–	10.5
S. albus	Eye swab	31	16	16	10	10	13	15
S. albus	Eye swab	29	20	17	10	–	–	–
S. albus	Eye swab	31	18	16	10	10	–	–
S. albus	Eye swab	–	–	–	–	–	14.5	–
S. albus	Wound swab	32	18	18	–	–	11	16
S. albus	Wound swab	22	21	18	15	13	11.5	–
S. albus	Wound swab	9	6	6	–	–	–	–
S. albus	Wound biopsy	11	11	10	–	–	9.5	21
S. albus	Wound swab	33	30	10	–	–	18	12

Key: 0 = undiluted honey
1:2 to 1:8 = aqueous dilutions of honey

Table III - Relative percentage resistance of *Staphylococcus* spp. to honey and the two standard antibiotics

Staph. spp	Honey	Antibiotic						
	0*	1:2	1:4	1:6	1:8	1:10	Cloxacillin (5 μg/ml)	Ampicillin (10 μg/ml)
S. aureus	3.3%	6.6%	13.3%	73.3%	86.6%	100%	36.6%	60%
S. albus	5.0%	5.0%	5.0%	4.0%	50%	80%	50%	55%

0* = undiluted honey

terial activity, followed by 93.3% for 1:2 dilution, and 86.6% for 1:4 dilution against *S. aureus*. Against *S. albus*, undiluted honey gave respectively 95%, 95%, 60%, and 50% of antibacterial activity. Two strains of the two *Staphylococcus* spp. (SA_6 and SB_{18}) showed total resistance to honey (*Table III*). With respect to the two standard antibiotics, 63.3% of *S. aureus* isolates and 50% of *S. albus* isolates were sensitive to cloxacillin, in sharp contrast to the 40% and 45% of *S. aureus* and *S. albus*, respectively, against ampicillin. Of the 23 isolates of *S. aureus* that showed resistance to either of the two antibiotics, only six (i.e. 26%) were resistant to both antibiotics. With *S. albus*, eight strains (66.6%) of the 12 similarly resistant isolates were resistant to both antibiotics. *Table III* shows the relative percentage resistance of the two *Staphylococcus* spp. to honey and the two standard antibiotics.

Discussion and conclusion

The antistaphylococcal activity recorded in this study for honey was consistent with earlier reports on its curative and antibacterial properties.[11,14,16,19,20] *S. albus* showed a resistance level of 5.0% to undiluted honey and of 3.3% to *S. aureus*. Zones of growth inhibition of 10 mm or more for 50% and 75% dilutions of honey in agar-cup diffusion as the basis for offering honey as a unique treatment in staphylococcal infections[16] found support in this study.

Compared with honey and its 1:2 to 1:6 dilutions, cloxacillin and ampicillin used in concentrations of 5 μg/ml and 10 μg/ml were less active on both *S. aureus* and *S. albus*. The resistance levels among the *S. aureus* isolates were respectively 36.6% and 60% for cloxacillin and ampicillin compared with 3.3%, 6.6%, and 13.3% for undiluted honey, 1:2, and 1:4 dilutions respectively. With *S. albus*, the picture was similar. These results find an analogy in an earlier report[14] of more effective treatment of bacterial infection with honey in burn wounds than with silver sulphadiazine, a recognized antibacterial ointment.

This study buttresses the potential of honey as a suitable therapeutic agent against bacterial infections, particularly those due to resistant staphylococci, in the search for a solution to the problem of antibiotic resistant strains.

RÉSUMÉ. Cinquante isolats bactériens composés de *Staphylococcus aureus* et de *Staphylococcus albus* provenant de six sources pathologiques ont été exposés au miel et à ses dilutions fractionnelles comme aussi à deux antibiotiques standard - cloxacilline (5 mg/ml) et ampicilline (10 mg/ml) - dans un test de sensibilité de diffusion «agar-cup». Le miel non dilué présentait une activité antibactérienne à presque 100% des isolats bactériens tandis que ses dilutions fractionnelles de 1:2 jusqu'à 1:10 possédaient des propriétés antibactériennes contre les isolats en ordre décroissant d'activité. La cloxacilline présentait une activité antibactérienne plus élevée par rapport à l'ampicilline mais toutes les deux exerçaient une activité antibactérienne inférieure à celle du miel. L'application thérapeutique du miel, à la suite des épreuves cliniques, devrait être importante dans le traitement des infections bactériennes et en particulier les infections causées par les deux espèces de *Staphylococcus* testées.

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