

INCIDENCE AND ANTIBIOTIC SENSITIVITY OF STAPHYLOCOCCI IN INFECTED EAR, NOSE AND THROAT

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Abstract

Five hundred and ten samples taken from patients suffering from infections of ear, nose and throat (E.N.T.) were studied in the Department of Microbiology, University of Agriculture, Faisalabad. One hundred and seventy samples taken from each source revealed an incidence of *Staphylococcus aureus* 53.5 per cent, *Staphylococcus epidermidis* 16.5 per cent and *Staphylococcus saprophyticus* 1.4 per cent. Pathogenicity trials of randomly selected 24 strains of *Staphylococcus aureus* were carried out in rabbits and mice. The *in vitro* antibiotic sensitivity test of *Staphylococcus aureus* against 11 antibiotics revealed gentamicin to be the most effective against ear and throat infections while vibramycin was effective against nasal infections. Amoxycillin, penicillin G and tetracyclin were the least effective drugs against all the isolates of *Staphylococcus aureus* from the three sources.

Introduction

Staphylococci are frequently encountered in the upper respiratory tract where they play a significant role in the initiations of infections of E.N.T. The problems created by Staphylococci are increasing day by day because even in the era of chemotherapy so many virulent strains have become resistant to antibiotics. This gloomy situation has arisen due to the extensive use of antibiotics prior to their sensitivity tests. Since different strains of Staphylococci differ in sensitivity to various antibiotics, the choice of antibiotics in treatment of a patient should be based on the results of sensitivity tests.

This study was, therefore, undertaken firstly to determine the incidence of Staphylococci in infected cases of E.N.T. and secondly to find the most effective antibiotics against the strains isolated.

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Material and Methods

Samples were collected from 510 patients suffering from various infections of ear, nose and throat (E.N.T.) attending District Headquarter Hospital and Dispensary of the University of Agriculture, Faisalabad from 1st September, 1976 to 30th August, 1977. One hundred and seventy samples each were taken from these three sources. They were cultured on different laboratory media for the primary isolation and the study of pigment production. The media used included Staphylococcus agar No. 110 and Mannitol Salt agar Difco Laboratories (1953), the haemolytic properties of the isolates were studied by using blood agar containing 10 per cent defibrinated sheep blood.

The pure isolates were then characterised and identified by performing morphological, biochemical and other bacteriological tests (Cruikshank et al., 1975).

For further identification of the test strains, 24 strains of *Staphylococcus aureus* were selected randomly and their pathogenicity trials were carried out in rabbits and mice. The methods described by Cohn (1962) and Li and Kapral (1962) were followed.

The *in vitro* antibiotic sensitivity tests of all the isolates of *Staphylococcus aureus* was performed on blood agar using 11 antibiotics. The method described by Barry et al. (1970) was followed.

All the isolates of Staphylococci were lyophilized and their viability was checked after 3 months.

Results

Studies on the 510 samples taken from patients suffering from various infections of

E.N.T. revealed the total occurrence of *Staphylococcus aureus* to be 273 (53.5 per cent), *Staphylococcus epidermidis* 84 (16.5 per cent) and *Staphylococcus saprophyticus* 7 (1.4 per cent). From the 170 samples, taken from infections of ear *Staphylococcus aureus* was isolated from 73 (42.9 per cent), *Staphylococcus epidermidis* 26 (15.2 per cent) and *Staphylococcus saprophyticus* 7 (4.1 per cent) cases. One hundred and seventy nasal cultures revealed the presence of *Staphylococcus aureus* in 104 (61.1 per cent) and *Staphylococcus epidermidis* 43 (25.2 per cent) cases. One hundred and seventy throat cultures revealed the presence of *Staphylococcus aureus* in 96 (56.4 per cent) and *Staphylococcus epidermidis* in 15 (8.8 per cent) cases. *Staphylococcus saprophyticus* could not be isolated either from nose or throat infections. The site of lesions and type of Staphylococci showed a significant correlation $X^2=9.277$ (Table I).

Table I: Incidence of Staphylococci in Ear, Nose and Throat (E.N.T.)

Source	No. of samples	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus saprophyticus</i>
Ear	170	73 (42.9%)	26 (15.2%)	7 (4.1%)
Nose	170	104 (61.1%)	43 (25.2%)	—
Throat	170	96 (56.4%)	15 (8.8%)	—

$X^2=9.2772$.

The 24 strains of *Staphylococcus aureus* which were selected randomly (8 from each source) proved pathogenic for 20 out of 24 rabbits inoculated intravenously. The death of rabbits was recorded between 18-48 hours post inoculation (HPI). The details of the mortality rates are shown in table II.

Similarly the 48 mice inoculated by the same route with 24 strains died within 9-27 HPI as shown in table III.

Table II: Pathogenicity Trials in Rabbits

Group	Source	Rabbits inoculated	Mortality rate (hours post inoculation (HPI))						
			6-12	12-18	18-24	24-30	30-36	36-42	42-48
A	Ear	8	—	1	1	2	2	2	—
B	Nose	8	—	—	1	2	2	1	—
C	Throat	8	—	—	—	2	1	2	1

Table III: Pathogenicity Trials in Mice

Group	Source	Mice inoculated	Mortality rate (Hours post inoculation (HPI))								
			0-3	3-6	6-9	9-12	12-15	15-18	18-21	21-24	24-27
A	Ear	16	—	—	—	4	8	—	4	—	—
B	Nose	16	—	—	—	4	2	4	4	2	—
C	Throat	16	—	—	—	4	2	2	4	2	2

The *in vitro* antibiotic sensitivity results of all the isolates of *Staphylococcus aureus* are summarised in tables IV, V and VI. The drugs are tabulated in declining order of sensitivity. In addition to the percentage of cases sensitive levels of +++ and +++ taken as positive for sensitivity for various drugs, a more comprehensive calculations showing mean sensitivity score \pm S.D. are also shown in these tables.

Table IV: Antibiotic Sensitivity of 73 Isolates of *Staphylococcus Aureus* (Ear Infection)

Sensitivity	Percentage Sensitivity*	Sensitivity Score \pm S.D.
Gentamycin	64/73(87.6)	3.45 \pm 0.85
Septtran	45/73(61.64)	2.84 \pm 1.32
Vibramycin	38/73(52.05)	2.38 \pm 1.25
Cloxacillin	23/73(31.51)	1.81 \pm 1.23
Chloramphenicol	28/73(38.36)	2.01 \pm 1.5
Erythromycin	27/73(36.99)	2.27 \pm 1.40
Ampiclox	25/73(34.25)	2.08 \pm 1.52
Combiotic	10/73(13.70)	1.48 \pm 1.26
Penicillin	4/73(5.48)	0.82 \pm 1.08
Tetracycline	6/73(8.22)	0.93 \pm 1.12
Amoxycillin	19/73(26.03)	1.48 \pm 1.32

*Sensitivity level of +++ and +++ were taken as positive.
Figures in parenthesis indicate percentage positive.

Table V: Antibiotic Sensitivity of 104 Isolates of *Staphylococcus Aureus* (Nasal Infection)

Sensitivity	Percentage sensitivity*	Sensitivity score \pm S.D.
Vibramcin	97/104(91.3)	3.64 \pm 0.87
Gentamycin	85/104(81.73)	2.45 \pm 1.05
Chloramphenicol	66/104(63.46)	2.59 \pm 1.29
Combiotic	60/104(57.69)	2.23 \pm 1.22
Cloxacillin	54/104(51.92)	2.16 \pm 1.29
Septtran	52/104(50.00)	2.04 \pm 1.60
Ampiclox	43/104(41.35)	1.80 \pm 1.84
Erythromycin	42/104(40.38)	1.73 \pm 1.62
Amoxycillin	38/104(36.54)	1.69 \pm 1.56
Tetracycline	23/104(22.12)	1.02 \pm 1.34
Penicillin G	19/104(18.27)	0.98 \pm 1.24

*Sensitivity level of +++ and +++ were taken as positive.
Figures in parenthesis indicate percentage positive.

Table VI: Antibiotic Sensitivity of 96 Isolates of *Staphylococcus Aureus* (Throat Infection)

Sensitivity	Percentage sensitivity*	Sensitivity score \pm S.D.
Gentamycin	68/96(70.8)	2.89 \pm 1.18
Chloramphenicol	62/96(64.58)	2.37 \pm 1.54
Septtran	57/96(59.38)	2.71 \pm 1.65
Combiotic	53/96(55.21)	2.38 \pm 1.54
Erythromycin	42/96(43.75)	2.36 \pm 1.54
Vibramycin	39/96(40.63)	3.06 \pm 1.75
Amoxycillin	31/96(32.29)	2.99 \pm 1.73
Cloxacillin	28/96(29.17)	2.62 \pm 1.62
Ampiclox	20/96(20.83)	2.57 \pm 1.60
Penicillin G	11/96(11.46)	1.50 \pm 1.22
Tetracycline	6/96(6.25)	0.88 \pm 0.93

*Sensitivity level of +++ and +++ were taken as positive.
Figures in parenthesis indicate percentage positive.

All the lyophilized isolates of *Staphylococci* remained viable even after a period of 3 months.

Discussion

In the current study the total occurrence of *Staphylococcus aureus* in infected E.N.T. was 273 (53.5 per cent), *Staphylococcus epidermidis* was 84 (16.4 per cent) and *Staphylococcus saprophyticus* was 7 (1.4 per cent) $\chi^2=9.27$ (Table I). These results are in agreement with those of Wilson and Alkinson (1945), Qureshy and Hafiz (1973) who observed that *Staphylococci* are most frequently associated with the infections of upper respiratory tract.

The occurrence of *Staphylococcus aureus* in ear infections was 42.9 per cent and this was observed to be lower than that in nose and throat infections. These results are in total agreement with those of Merchant and Packer (1966). In the present investigation, the occurrence of *Staphylococcus aureus* in nasal cultures was 61.1 per cent i.e. 104 out of 170 samples were positive for the growth of this organism. These results are in accordance with the investigations of Maouis et al. (1969) who also isolated 47 per cent of *Staphylococcus aureus* from the nasopharynxes of 136 infants suffering from *Staphylococcal pneumonia* but the findings of Stokes et al. (1970) differ significantly from the present study. The difference in results may be due to the difference in the type of medium used for the primary isolation. They had used blood agar whereas in the present research selective media were employed i.e. *Staphylococcus* agar No. 110 and Mannitol salt agar.

Among the throat infections included in the present study, the occurrence of *Staphylococcus aureus* was 56.4 per cent i.e. it was isolated from 96 out of 170 cases. This incidence differs somewhat from the study of Qureshy and Hafiz (1973). This difference in results may be due to the fact that in the present study *Staphylococci* were isolated from various types of the infections of the throat, whereas these workers studied only the cases of tonsillitis.

In the present study it was observed that gentamycin was the most effective drug against isolates from ear infections, it was found effective against 87.6 per cent of the strains of *Staphylococcus aureus* with sensitivity score 3.45 ± 0.80 (Table IV). This is in agreement with the findings of Federspil (1969) and Afridi (1977) who had found gentamycin to be effective against 87 per cent of the cases of E.N.T. diseases and otitis media respectively. Penicillin and tetracycline were least effective against the *Staphylococcus aureus* isolated from the ear infections (12.3 per cent and 8.2 per cent respectively). These results are comparable to those of Farooki (1965) and

Wysocki and Druner (1971) who recorded 18 per cent resistance of *Staphylococci* against tetracycline and 30-35 per cent sensitivity of *Staphylococci* to penicillin respectively.

The antibiotic most effective against the 104 strains of *Staphylococcus aureus* isolated from various nasal infections was vibramycin. It inhibited 91.3 per cent of the test strains with sensitivity score 3.64 ± 0.87 (Table V). These results are in total agreement with those of Agbin (1964) who observed that vibramycin showed significantly superior results as 90 per cent of the cases of acute and chronic sinusitis responded to this drug.

Gentamycin was most effective in 70.8 per cent with sensitivity score 2.89 ± 1.18 (Table VI) against the 96 strains of *Staphylococcus aureus* isolated from infections of throat. These results are in line with the reports of several workers such as Barber and Waterworth (1966) and Holt and Newman (1971). Penicillin G and tetracycline did not exhibit encouraging results and were sensitive to 11.4 per cent and 6.2 per cent only. These results are comparable to those of Barber (1947) who also reported a gross resistance of *Staphylococcus aureus* towards penicillin. This phenomenon may be explained on the basis of production of penicillins by the strains.

References

- Afridi, M.A.K. (1977) Management of chronic suppurative otitis media. *Rawal Med. J.*, 6:1.
- Agbin, O.G. (1974) A comparative trial of doxycycline (vibramycin) and ampicillin in the treatment of acute sinusitis. *Curr. Med. Res. Opin.*, 2:291.
- Barber, M. (1947) Staphylococcal infection due to penicillin resistant strains. *Br. Med. J.*, 2:863.
- Barber, M. and Waterworth, P.M. (1966) Activity of gentamycin against pseudomonas and hospital staphylococci. *Br. Med. J.*, 1:203.
- Barry, A.I., Garcia, F. and Thrupp, L.D. (1970) An improved single-disk method for testing the antibiotic susceptibility of rapidly growing pathogens. *Am. J. Clin. Path.*, 53:149.
- Cohn, Z.A. (1962) Determinate of infection in the peritoneal cavity. I. Response to and fate of *Staphylococcus aureus* and *Staphylococcus albus* in the mouse. *Yale. J. Biol. Med.*, 35:12.
- Cruickshank, R., Duguid, J.P., Marriott, B.P. and Swain, R.H.A. (1975) *Medical Microbiology*, Vol. II, Edinburgh, Churchill Livingstone, 1975.
- Difco Laboratories. *Difco Manual of dehydrated cultural Media and reagents for microbiological and clinical Laboratory procedures*. 9th ed. Michigan, Difco Laboratories, 1953.
- Farooqi, M.A. (1965) Observations on staphylococcal antibiotic resistance. *Medicus*, 29:170.
- Federspil, P. (1969) Clinical use of gentamycin in ear, nose and throat infections. *J. Inf. Dis.*, 119:465.

Holt, R.J. and Newman, R.L. (1971) Gentamycin in Pediatrics. II. Treatment of severe urinary tract infections caused by gram-negative bacilli and of staphylococcal infections. *J. Inf. Dis.*, 124 (Suppl.):257.

Li, I.W. and Kapral, F.A. (1962) Virulence and coagulases of *Staphylococcus aureus*. II. Survival of certain coagulase negative mutants in the organs of intravenously injected rabbits. *J. Infec. Dis.*, III:204.

Mazouis, F., Nicolopoulos, D., Papisideri, P. and Benetos, S. (1969) Staphylococcal pneumonia in infants. *Lancet*, 2:268.

Merchant, C.A. and Packer, R.A. *Veterinary bacteriology and virology*. 5th ed. Ames, Iowa, Iowa State College press, 1956, p. 245.

Ogreshy, F. and Hafiz, A. (1973) Bacterial flora of tonsillitis and healthy persons. *J.P.M.A.*, 23:301.

Stockes, E.R., Ingham and Selkon, J. B. (1970) A screening technique for the detection of nasal carriers of antibiotic-resistant *Staphylococcus aureus*. *J. Clin. Path.*, 23:346.

Wilson, G.S. and Atkinson, J.D. (1945) Typing of staphylococci by the bacteriophage method. *Lancet*, 1:647.

Wysocki, S. and Druner, H.U. (1971) *The changing pattern of infecting organisms*. Bayer Symposium Bacterial Infections Springer-Verlag Berlin, Heidelberg, N.Y. 3:25.