Gram-positive, motile, cluster-forming cocci as a cause of urinary infection

S. VIRTANEN¹

From the National Public Health Laboratory, Turku, Finland

SYNOPSIS One hundred and thirteen strains of motile, Gram-positive, catalase-positive, cluster-forming cocci were isolated from patients with urinary infection attending a private surgery. They constituted 1% of the total 11 302 positive cultures. The biochemical characteristics and the drug sensitivities of the strains are described. The significance of motility for organisms which cause urinary infections is pointed out.

At the present time the organisms isolated are orphans in the controversial classification of staphylococci and micrococci.

The classification and nomenclature of staphylococci and micrococci is one of the most confusing examples of taxonomy in microbiology (Baird-Parker, 1965a). The traditional classification according to Bergey's manual (Breed, Murray, and Smith, 1957) has been found unsatisfactory (Shaw, Stitt, and Cowan, 1951; Thatcher and Simon, 1957; Garvie, Higgs, and Neave, 1961; Pohja and Gyllenberg, 1962). Numerous new schemes have been proposed (Shaw *et al.*, 1951; Baird-Parker, 1962, 1963, 1965b, and 1966; Gibson, 1967). These suggestions, with their numerous groups, subgroups, and biotypes, have not been sufficient to establish a good general classification. One of the most successful schemes was later labelled as a bad one by the author who constructed it (Cowan, 1971). It is generally agreed that there is a need to separate the mainly parasitic and facultative anaerobic staphylococci from the mainly saprophytic and aerobic micrococci. The problem remains, however, as to the best methods of separation (Baird-Parker, 1965a).

The Subcommittee on Taxonomy of Staphylococci and Micrococci (International Committee on Nomenclature of Bacteria, 1971) favoured the acceptance of four genera of Gram-positive, catalase-positive, and cluster-forming cocci: *Staphylococcus rosenbach*, *Micrococcus cohn*, *Planococcus migula*, and *Aerococcus williams*, *hirsch*, and *cowan*. The genus *Aerococcus* includes the organisms previously

placed in the genera *Aerococcus* and *Gaffkya*. Although the importance of the oxidation-fermentation (F/O) test has been criticized (Gibson, 1967; Mortensen and Kocur, 1967; Møller, Christiansen, and Mortensen, 1973), the Subcommittee considered the standard O/F test still the most reliable method for distinguishing between micrococci and staphylococci. The value of DNA analysis (Mortensen and Kocur, 1967) was considered to need further research.

The coagulase-negative, cluster-forming cocci have been neglected by medical bacteriologists in the past largely because most pyogenic lesions were caused by coagulase-positive *Staphylococcus aureus* strains, and partly because of the lack of any generally accepted criteria for distinguishing the seemingly numerous varieties of coagulase-negative staphylococci and micrococci. Although these organisms were long recognized as a rare and exceptional cause of infection, it is precisely in recent years that more attention has been paid to their role in various infections (Quinn, Cox, and Fisher, 1965; Person, Yu, and Washington, 1969; Corse and Williams, 1968; Speller and Mitchell, 1973). The organisms have sometimes been called *Staphylococcus albus* or *Staphylococcus epidermidis*, sometimes coagulase-negative staphylococci or micrococci.

These strains have been found to be associated with urinary tract infections (Torres Pereira, 1962; Roberts, 1967; Mitchell, 1968; Mabeck, 1969a). They have been reported to be causative agents in 4 to 14% of urinary infections (Mabeck, 1969b; Gallagher, Montgomerie, and North, 1965; Torres Pereira, 1962).

¹Present address: Kansanterveyslaboratorio aluelaboratorio Künämyllynkatu 10, 20520 Turku 52, Finland.

Material and Methods

Thirty-three thousand nine hundred clean-caught midstream voided urine samples were studied at a private surgery in the years 1969-1973. The urine samples were obtained after carefully washing the external genitalia with soap and sterilized water. Eleven thousand three hundred and two of the cultures were positive. In 113 cases Gram-positive, catalase-positive, phosphatase-negative, coagulase-negative, cluster-forming cocci were isolated. Ninety-four of the patients were women and 19 were men. The ages of the women varied from 15 to 81 years (mean 34.8 ± 1.8 years); that of the men varied from 24 to 75 years (mean 58.8 ± 1.1 years).

A wet mount of the sample was immediately microscoped for the presence of bacteria and pus cells. Three or more leucocytes per high-power field were considered to indicate pyuria. The sample was Gram-stained and cultured with calibrated loops on a bromocresol purple lactose agar plate, on a 5% sheep's blood agar plate, and after dilution with saline to 10^{-5} on a nutrient agar plate. The loops were calibrated to deliver 0.01 ml and 0.001 ml respectively. A colony count of over 10^5 bacteria/ml was taken to indicate true bacteriuria.

The drug sensitivities were determined by Ericsson's disc technique (Ericsson and Sherris, 1971). The discs employed were commercial ones (Biodisc Co), except the 5 µg novobiocin discs (Mitchell and Baird-Parker, 1967), which were prepared by the author.

The tests for the free coagulase, and the O/F test for the anaerobic production of acid from glucose were carried out as suggested by the Subcommittee on Taxonomy of Staphylococci and Micrococci (Baird-Parker, 1965c). Urease activity was studied in the Difco (B 272) medium using a heavy inoculum, DNA-ase according to Mortensen and Kocur (1967), and the other biochemical tests were done according to Baird-Parker (1963).

The pigment production was observed first after 24 hours incubation at 37°C, and later after two days at room temperature.

The motility was observed in 'hanging-drop' preparations of young cultures in nutrient broth, and checked in Graigie tubes with 0.1% nutrient agar (Cruickshank, 1969). For the demonstration of flagella Bailey's flagella stain was employed (Society of American Bacteriologists 1957).

Results

The results of the colony counts and microscopic examination are given in table I. Pyuria was demon-

Microscopic Examination	Bacterial Count (organisms/ml)		Total
	$10^4 - 10^5$	$>10^5 - \geq 10^7$	
Pyuria +	8	34	42
Pyuria -	12	59	71
	20	93	113

Table I The results of colony counts

	Positive	Negative	Total
Catalase	113	—	113
Coagulase	—	113	113
DNAase	7	106	113
Egg yolk	7	106	113
Phosphatase	—	113	113
Urease	113	—	113
Assimilation of $\text{NH}_4\text{H}_2\text{PO}_4$	5	108	113
Growth at 44°C	62	51	113
Growth after 1/2 hr at 56°C	17	96	113
Growth on 40% bile agar	113	—	113
Growth in 10% NaCl broth	113	—	113
Motility	113	—	113
Oxidation of glucose	113	—	113
Fermentation of glucose	33	80	113
Aerobic acid production from glucose	113	—	113
Aerobic gas production from glucose	—	113	113
Aerobic acid production from:			
Arabinose	—	113	113
Galactose	113	—	113
Inositol	—	113	113
Lactose	113	—	113
Maltose	113	—	113
Mannitol	75	38	113
Salicin	8	105	113
Gelatine liquefaction	44	69	113
Hippurate hydrolysis	76	37	113
Voges-Proskauer test	111	2	113

Table II Biochemical tests and growth characteristics

	<i>Sensitive</i>	<i>Moderately Resistant</i>	<i>Resistant</i>	<i>Total</i>
Ampicillin	106	6	1	113
Chloramphenicol	106	1	6	113
Erythromycin	110	1	2	113
Nalidix acid	3	—	110	113
Nitrofurantoin	113	—	—	113
Novobiocin	113	—	—	113
Penicillin	110	3	—	113
Sulphonamide	68	7	38	113
Tetracycline	83	12	18	113

Table III *Drug sensitivities*

strated in 37.2%. Gram-positive cocci in clusters were always seen in the direct smears made immediately after voiding.

Slightly yellowish pigment was formed by 25 strains; 88 strains were ivory. All strains were motile. The motility in culture media employed was not as fast as that of enterobacteria, but was generally demonstrated within a week. Young cultures of

recently isolated strains showed a lively motility in 'hanging-drop' preparations.

The growth characteristics and the results of biochemical tests are shown in table II, and drug sensitivities in table III.

Motile cultures examined by the Bailey stain showed 1-2 flagella (fig 1). An electron micrograph is shown in figure 2.

Discussion

The occurrence of bacteria in direct mounts and Gram-stained smears and high colony counts of a single strain, fairly frequent pyuria, and the simultaneously occurring clinical symptoms justify with reasonable certainty regarding these organisms as causative agents of infection. Only one sample was obtained from each patient, because the treatment could not be postponed for dysuria, frequent urination, or other clinical symptoms and signs.

The Gram-positive, catalase-positive, and cluster-forming cocci isolated from the cases of urinary infection do not seem to constitute a homogeneous group. Their biochemical characteristics and their drug sensitivities vary. However, the two common characteristics (motility and urease activity) might be useful for an organism causing urinary infection. The vast majority of urinary infections are caused by motile microorganisms ascending the urinary passages, eg, enterobacteria and enterococci.

From the taxonomical point of view the organisms isolated present a dilemma. They do not fit any of the groups presently proposed (International Committee on Nomenclature of Bacteria, 1971) for the classification of micrococci and staphylococci. At present the motile micrococci are placed in the genus *Planococcus migula*. The characteristics of the genus *Planococcus migula* were rather vaguely described by the original author (Migula, 1894 and 1900). Kocur, Pácoba, Hodgkiss, and Martinez (1970) described in more detail the characteristics of the flagellated marine cocci placed in the genus *Planococcus*. Their strains did not produce acid from glucose, did not

Fig 1 *Flagellated cluster-forming cocci. Staining by Bailey's method, 1200.*

Fig 2 Electron micrograph of a flagellated coccus. Negatively stained with phosphotungstic acid, 40 500.

produce acetoin, and were distinctly pigmented. The strains isolated by the author do not correspond to any of the seven species described by Migula (1900). At present the organisms are orphans in the taxonomy of staphylococci and micrococci.

The organisms studied were isolated in the course of five years from 33 900 samples taken from out-patients. Eleven-thousand three-hundred and three of the samples were positive on culture, and the vast majority were various enterobacteria. Thus, the organisms of the present study constitute only 1% of the total. Consequently they seem to be a rare cause of urinary infection, and therefore may be sometimes wrongly regarded as unimportant contaminants. This is especially the case when they occur simultaneously with contaminant flora. A meticulous and careful technique in obtaining urine samples is extremely important and cannot be overemphasized.

Gram-positive, catalase-positive, and cluster-forming cocci have been isolated from the cases of urinary infection by several authors (Mortensen, 1969; Roberts, 1967; Mitchell, 1968; etc). They form a heterogeneous group containing staphylococci and micrococci. The strains reported by Mortensen (1969) were non-motile, but usually the motility has not been mentioned by the authors.

Obviously the strains isolated do not fit well into the present taxonomy but do so better *in vivo*. Anyway they form a 'minority group' which is easily overlooked. It is likely that their small but definite role will be more clearly defined in the future.

References

- Baird-Parker, A. C. (1962). The occurrence and enumeration, according to a new classification, of micrococci and staphylococci in bacon and on human and pig skin. *J. appl. Bact.*, **25**, 352-361.
- Baird-Parker, A. C. (1963). A classification of micrococci and staphylococci based on physiological and biochemical tests. *J. gen. Microbiol.*, **30**, 409-427.
- Baird-Parker, A. C. (1965a). Staphylococci and their classification. *Ann. N.Y. Acad. Sci.*, **128**, 4-25.
- Baird-Parker, A. C. (1965b). The classification of staphylococci and micrococci from world-wide sources. *J. gen. Microbiol.*, **38**, 363-387.
- Baird-Parker, A. C. (1965c). Recommendations of the Subcommittee on taxonomy of staphylococci and micrococci. *Int. Bull. bact. Nomencl.*, **15**, 109-110.
- Baird-Parker, A. C. (1966). Methods for classifying staphylococci and micrococci. In *Identification methods for Microbiologists*, Pt A (Society for Applied Bacteriology, Technical Series, No. 1), edited by B. M. Gibbs and F. A. Skinner, pp. 59-64. Academic Press, London and New York.
- Breed, R. S., Murray, E. G. D., and Smith, N. R. (1957). *Bergey's Manual of Determinative Bacteriology*, 7th ed. Bailliere, Tindall, and Cox, London.
- Corse, J., and Williams, R. E. O. (1968). Antibiotic resistance of coagulase-negative staphylococci and micrococci. *J. clin. Path.*, **21**, 722-728.
- Cowan, S. T. (1971). Sense and nonsense in bacterial taxonomy. *J. gen. Microbiol.*, **67**, 1-8.
- Cruickshank, R., ed., (1969). *Medical Microbiology*, 11th ed., p. 235. Livingstone, Edinburgh and London.
- Ericsson, H. M., and Sherris, J. C. (1971). Antibiotic sensitivity testing. *Acta path. microbiol. scand.*, Sect. B, Suppl. 217.
- Gallagher, D. J. A., Montgomerie, J. Z., and North, J. D. K. (1965). Acute infections of the urinary tract and the urethral syndrome in general practice. *Brit. med. J.*, **1**, 622-626.
- Garvie, E. I., Higgs, T. M., and Neave, F. K. (1961). Unusual varieties of *Staphylococcus aureus*. *J. Path. Bact.*, **81**, 255-259.
- Gibson, T. (1967). The status of the genus *Micrococcus*. *Int. J. system. Bact.*, **17**, 231-233.
- International Committee on Nomenclature of Bacteria, Subcommittee on Taxonomy of Staphylococci and Micrococci (1971). Minutes of meeting held 2/3 April 1968. *Int. J. system. Bact.*, **21**, 161-164.
- Kocur, M., Páčova, Z., Hodgkiss, W., and Martinec, T. (1970). The taxonomic status of the genus *Planococcus* Migula 1894. *Int. J. system. Bact.*, **20**, 241-248.
- Mabeck, C. E. (1969a). Studies in urinary tract infections. II. Urinary tract infection due to coagulase-negative staphylococci. *Acta med. scand.*, **186**, 39-45.
- Mabeck, C. E. (1969b). Significance of coagulase-negative staphylococcal bacteriuria. *Lancet*, **2**, 1150-1152.
- Migula, W. (1894). Ueber ein neues System der Bakterien. *Arb. bakt. Inst. Karlsruhe*, **1**, 235-238.
- Migula, W. (1900). *System der Bakterien*, 269. Fischer, Jena.
- Mitchell, R. G. (1968). Classification of *Staphylococcus albus* strains isolated from the urinary tract. *J. clin. Path.*, **21**, 93-96.
- Mitchell, R. G., and Baird-Parker, A. C. (1967). Novobiocin resistance and the classification of staphylococci and micrococci. *J. appl. Bact.*, **30**, 251-254.

- Mortensen, N. (1969). Studies in urinary tract infections III. Biochemical characteristics of coagulase-negative staphylococci associated with urinary tract infections. *Acta med. scand.* **186**, 47-51.
- Mortensen, N., and Kocur, M. (1967). Correlation of DNA base composition and acid formation from glucose of staphylococci and micrococci. *Acta. path. microbiol. scand.*, **69**, 445-457.
- Møller, J. K., Christiansen, C., and Mortensen, N. (1973). DNA base composition of coagulase negative staphylococci associated with urinary tract infection. *Acta path. microbiol. scand.*, Sect. B., **81**, 559-562.
- Person, D. A., Yu, P. K. W., and Washington, J. A. II (1969). Characterization of Micrococcaceae isolated from clinical sources. *Appl. Microbiol.*, **18**, 95-97.
- Pohja, M. S., and Gyllenberg, H. G. (1962). Numerical taxonomy of micrococci of fermented meat origin. *J. appl. Bact.*, **25**, 341-351.
- Quinn, E. L., Cox, F., and Fisher, M. (1965). The problem of associating coagulase-negative staphylococci with disease. *Ann. N.Y. Acad. Sci.*, **128**, 428-442.
- Roberts, A. P. (1967). Micrococcaceae from the urinary tract in pregnancy. *J. clin. Path.*, **20**, 631-632.
- Shaw, C., Stitt, J. M., and Cowan, S. T. (1951). Staphylococci and their classification. *J. gen. Microbiol.*, **5**, 1010-1023.
- Society of American Bacteriologists (1957). *Manual of Microbiological Methods*, pp. 27-28. McGraw-Hill, New York.
- Speller, D. C. E., and Mitchell, R. G. (1973). Coagulase negative staphylococci causing endocarditis after cardiac surgery. *J. clin. Path.*, **26**, 517-522.
- Thatcher, F. S., and Simon, W. (1957). Some physiological and toxic properties of members of the genus *Micrococcus* in relation to taxonomy. *Int. Bull. bact. Nomencl.*, **7**, 21-36.
- Torres Pereira, A. (1962). Coagulase-negative strains of staphylococci possessing antigen 51 as agents of urinary infection. *J. clin. Path.*, **15**, 252-253.

The April 1974 Issue

THE APRIL 1974 ISSUE CONTAINS THE FOLLOWING PAPERS

- Chemotherapy of experimental streptococcal endocarditis Part III Failure of a bacteriostatic agent (tetracycline) in prophylaxis FREDERICK S. SOUTHWICK AND DAVID T. DURACK
- An improved test for *Haemophilus influenzae* precipitins in the serum of patients with chronic respiratory disease JUDITH L. DAVIES, CHRISTIAN R. LAUGHTON, AND J. ROBERT MAY
- The effect of minocycline on *Candida albicans* PAMELA M. WATERWORTH
- An experimental assessment of different anaerobic blood culture methods D. C. SHANSON
- A comparison of media used *in vitro* to isolate non-sporing Gram-negative anaerobes from blood W. R. FORGAN-SMITH AND J. H. DARRELL
- Chlorhexidine resistance in *Proteus mirabilis* D. J. STICKLER
- Membrane filtration in the study of antimicrobial drugs P. D. MEERS AND GILLIAN M. CHURCHER
- A comparative study of viruses associated with acute haemorrhagic conjunctivitis P. G. HIGGINS, R. J. SCOTT, PATRICIA M. DAVIES, AND D. R. GAMBLE
- The relationship between urinary infection, cystoscopic appearance, and pathology of the bladder in man
I Lymphocytes in the lamina propria
II Squamous change in the bladder epithelium
F. P. MARSH, R. BANERJEE, AND P. PANCHAMIA
- Disseminated histoplasmosis successfully treated with amphotericin B R. P. S. EDMONSON, SUSANNAH EYKYN, D. R. DAVIES, I. W. FAWCETT, AND IAN PHILLIPS
- Mucin histochemistry of mucous carcinomas of breast and colon and non-neoplastic breast epithelium D. J. COOPER
- Changes in composition of mucin in the mucosa adjacent to carcinoma of the colon as compared with the normal: A biochemical investigation M. I. FILIPE AND K. B. COOKE
- Iodine contamination of the serum protein-bound iodine: Incidence and clinical significance P. R. PANNALL, P. C. MINNAAR, AND A. T. NESER
- Antibody to hepatitis B antigen in haemophiliacs and their household contacts C. J. BURRELL, A. C. PARKER, D. M. RAMSAY, AND ELAINE PROUDFOOT
- The effect of age on the fibrinolytic enzyme system P. J. HAMILTON, AUDREY A. DAWSON, D. OGSTON, AND A. S. DOUGLAS
- A system for data processing in haematology I. CAVILL, C. RICKETTS, T. MOULDING, A. JACOBS, AND M. PAGE
- Training of pathologists by a system of modules JOHN STUART AND PHILIP A. GORDON
- Technical method*
Demonstration lymphocyte nucleoli J. C. RIDWAY AND J. V. GARRETT
- Letter to the Editor
- Book reviews

Copies are still available and may be obtained from the PUBLISHING MANAGER,
BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON, WC1H 9JR, price £1.05.