

The Medical Significance and Identification of Coagulase - Negative Staphylococci

Introduction

To date over 40 species of the genus *Staphylococcus* have been described (1,2). The species most commonly implicated as the etiological agent in infections of humans and animals is the coagulase-positive *S. aureus*. The coagulase-negative staphylococci (CNS) are, as a group the most frequently encountered bacteria in medical microbiology laboratories (3,4) and have been considered to be saprophytic and rarely pathogenic. More recently however, CNS have emerged as significant pathogens, particularly in infections associated with medical devices and in immunocompromised patients. In early studies, *S. epidermidis*, *S. saprophyticus* and *S. haemolyticus* were associated with human infections (5,6). More recently numerous reports have implicated a much wider range of species as etiological agents associated with confirmed infectious processes, Table 1 (1,7,8).

S. epidermidis
S. saprophyticus
S. haemolyticus
S. capitis
S. hominis
S. lugdunensis
S. warneri
S. saccharolyticus
S. xylosus

Table 1. CNS implicated in human infectious disease.

The factors associating the CNS as etiological agents include (i) the isolation of strains in pure culture from infected sites or body fluids and (ii) the repeated isolation of the same strain(s) over the course of an infection (8). *S. epidermidis* is extremely well documented as a pathogen in cases of bacteraemia, prosthetic valve endocarditis, urinary tract, cerebrospinal fluid, peritoneal fluid and prosthetic joint infections. *S. haemolyticus* is the second most commonly encountered CNS associated with human infection, being implicated in cases of native – valve endocarditis, septicaemia, wound, bone and joint infections and urinary tract infections. *S. saprophyticus* is also well recognised as an opportunistic pathogen in urinary tract infections, particularly in young sexually active females. *S. lugdunensis* is also implicated in native- valve infections as well as arthritis, catheter, prosthetic joint and urinary tract infections.

The other species of CNS implicated in human infections (Table 1.) have been associated with endocarditis, septicaemia, pneumonia, wound and joint infections and osteomyelitis.

The Need for Identification

Despite this increased recognition of the significance of infections attributed to CNS, these organisms are not generally identified in medical microbiology laboratories. In most laboratory situations, the identification of staphylococci is restricted to colonial morphology, gram stain characteristics, catalase and coagulase (slide or tube), the latter largely being replaced by latex agglutination tests

(Microscreen Staph (M43)) in recent times. The application of these basic tests only permitting the classification of staphylococci into *S. aureus* and non- *S. aureus*, this latter group generally being referred to incorrectly as *S. epidermidis*.

The accurate identification of CNS is necessary in order that early predictions of potential pathogenicity and/ or antibiotic sensitivity can be made and the potential medical significance determined. In addition, the identification of CNS repeatedly isolated from patients with active infections is essential in the determination of strain similarity.

Without the identification of medically significant CNS, information pertaining to the presence and significance of these organisms and their role as aetiological agents of infectious disease will not be developed and progressed.

Identification Methods

Despite the need for simplified methods for the identification of individual member comprising the CNS, the schemes proposed by Kloos and Schleifer (9) and later modified by Bannerman (1) have been the only conventional methods available to laboratories. These methods, requiring a large number of biochemical tests, were laborious and required a prolonged incubation period to achieve a meaningful result.

Over the last decade or so, several commercial systems for the rapid identification of staphylococci have been developed as alternatives to the conventional methods above. In many cases these systems proved to be unreliable due to their attempts to identify all known CNS (medical, veterinary etc.). In addition, many of these systems were not cost compatible with medical laboratory functions (10).

Microgen[®] Staph ID (MID-69) is the newest addition to the Microgen ID product range (currently Gram Negative Bacilli, *Listeria* spp. and *Bacillus* spp.), and provides a simple and reliable identification system for the identification of those species of *Staphylococcus* commonly encountered in medical laboratories. Many of these species are however of veterinary importance (Table 2).

Staphylococcus spp.

S. aureus subsp *aureus*
S. aureus subsp *anaerobius*
S. auricularis
S. caprae
S. capitis subsp *capitis*
S. capitis subsp *urealyticus*
S. carnosus
S. chromogenes
S. cohnii subsp *cohnii*
S. cohnii subsp *urealyticum*
S. epidermidis
S. haemolyticus
S. hominis subsp *hominis*
S. hominis subsp *novobiosepticus*
S. hyicus
S. intermedius
S. lentus
S. lugdunensis
S. saccharolyticus
S. saprophyticus
S. schleiferi subsp *schleiferi*
S. schleiferi subsp *coagulans*
S. sciuri
S. simulans
S. warneri
S. xylosus

Kocuria spp.

K. kristinae
K. rosea
K. carniphila

Kytococcus spp.

Ky. sedentarius

Micrococcus spp.

M. luteus
M. lylae

Table 2. Species of *Staphylococcus* identified using Microgen Staph ID

By focussing on the key medical species of *Staphylococcus*, Microgen Bioproducts has successfully developed a simple and accurate identification system based on a total of 15 conventional tests. These tests include 13 biochemical substrates plus coagulase/ latex agglutination and colonial morphology (Table 3.). The 13 biochemical substrates are housed in the standard 12 microwell format (Nitrate is incorporated into the Glucuronidase well allowing 13 tests to be provided in the 12 microwells) employed in all of the Microgen identification systems.

To perform an identification, a single colony of the isolate to be identified is emulsified in 3ml of Staph suspending medium supplied in the kit. At the same time, a coagulase or latex test is

performed. Approximately 100µl of this suspension is inoculated into each microwell of the test panel. After the inoculation of the microwells, sterile mineral oil is overlaid on wells 10 and 11. The inoculated test panel is sealed and incubated at 35 - 37°C for 18 – 24 hours. After incubation reagents are added to the appropriate microwells (Nitrate A & B – well 9, PYR – well 12). After allowing approximately 10 minutes for colour development the individual reactions are read with reference to the colour chart provided (Figure 1.)

The results are recorded on the report form provided and the 5 digit Octal Code is calculated and entered into the Microgen® Identification System Software and a result generated.

Sucrose
Trehalose
Mannitol
N-Acetyl Glucosamine
Mannose
Turanose
Alkaline Phosphatase
Glucosidase
Glucuronidase/ Nitrate
Urease
Arginine
PYR

Coagulase/ Latex Agglutination
Colony Pigment

Table 3. Substrates and other tests included in the Microgen Staph ID

Performance

A total of 108 strains of *Staphylococcus* spp. comprising strains from recognised culture collections and clinical isolates from various donor laboratories were identified using both the Microgen® Staph ID (MID-69) and the API Staph (20 substrates). The coagulase/ latex reaction required for the Microgen® Staph ID identification was performed using the Microscreen Staph (M43) from Microgen Bioproducts. Both systems were inoculated and incubated in accordance with the manufacturers instructions.

The Microgen® Staph ID confirmed the identity of 107 (99%), whilst the API Staph also

confirmed the identity of 107 (99%) of the isolates tested. The Microgen® Staph ID was unable to correctly identify 1 strain of *S. haemolyticus* and the API was unable to identify one strain of *S. xylosus*. The results of this trial clearly demonstrates that the Microgen® Staph ID employing 15 specific substrate reactions was able to accurately identify a wide range of species of *Staphylococcus*.

	Total Tested	MID-69	API Staph
<i>S. aureus</i>	45	45	45
<i>S. epidermidis</i>	19	19	19
<i>S. haemolyticus</i>	12	11	12
<i>S. simulans</i>	8	8	8
<i>S. capitis</i> subsp. <i>capitis</i>	3	3	3
<i>S. capitis</i> subsp. <i>urealyticus</i>	2	2	2
<i>S. warneri</i>	5	5	5
<i>S. saprophyticus</i>	2	2	2
<i>S. lugdunensis</i>	2	2	2
<i>S. chromogenes</i>	2	2	2
<i>S. hominis</i>	3	3	3
<i>S. cohnii</i> subsp. <i>cohnii</i>	1	1	1
<i>S. lentus</i>	1	1	1
<i>S. xylosus</i>	1	1	0
<i>S. caprae</i>	1	1	1
<i>S. auricularis</i>	1	1	1
Total	108	107	107

Table 4. Summary of Microgen ID Staph and API Staph identification results.



Figure 1. Microgen Staph ID identification test panel.

Key features of the Microgen® Staph ID include the use of a coagulase/latex test which will clearly separate *S. aureus* from the coagulase negative species such as *S. haemolyticus* and the inclusion of the PYR test which has been demonstrated to be a valuable addition in the differentiation. The development of a database containing all medically recognised and relevant Staphylococcal species and the careful selection of those tests and substrates most appropriate to the identification of these species has enabled Microgen Bioproducts to develop an extremely efficient and easy-to-use identification system for the identification of these organisms.

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