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Staphylococcus hominis subsp. *novobiosepticus* subsp. nov., a novel trehalose- and *N*-acetyl-D-glucosamine-negative, novobiocin- and multiple-antibiotic-resistant subspecies isolated from human blood cultures

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A new subspecies, *Staphylococcus hominis* subsp. *novobiosepticus*, isolated from human blood cultures, a wound, a breast abscess and a catheter tip, is described on the basis of a study of 26 strains isolated between 1989 and 1996. DNA–DNA reassociation reactions, conducted under stringent conditions, and macrorestriction pattern analysis demonstrated that these strains are closely related to previously characterized *S. hominis* strains isolated from human skin and clinical specimens, but are significantly divergent. *S. hominis* subsp. *novobiosepticus* can be distinguished from *S. hominis* (now named *S. hominis* subsp. *hominis*) by its combined characteristics of novobiocin resistance and failure to produce acid aerobically from D-trehalose and *N*-acetyl-D-glucosamine. Furthermore, all 26 strains of the new subspecies are resistant to nalidixic acid, penicillin G, oxacillin, kanamycin and streptomycin, and were either resistant or had intermediate resistance to methicillin and gentamicin. Most strains were also resistant to erythromycin, clindamycin, chloramphenicol, trimethoprim/sulfamethoxazole and ciprofloxacin. Based on a comparison of the sequences of a 1001 bp *mecA* amplification product from reference methicillin-resistant staphylococci, the *mecA* gene present in *S. hominis* subsp. *novobiosepticus* was identified as homologue A, commonly found in *S. aureus* and many coagulase-negative staphylococcal species. The type strain of *S. hominis* subsp. *novobiosepticus* is ATCC 700236^T. Descriptions of *S. hominis* subsp. *novobiosepticus* subsp. nov. and *S. hominis* subsp. *hominis* are given and the description of *S. hominis* is emended.

Keywords: *Staphylococcus hominis* subsp. *novobiosepticus* subsp. nov., *Staphylococcus hominis* subsp. *hominis*, multiple antibiotic resistance, *mecA* gene homologues, novobiocin-resistant *Staphylococcus hominis*

INTRODUCTION

Staphylococcus hominis is one of the major staphylococcal species inhabiting the skin of humans and, on most of the people that have been examined, it produces large populations in the axillae and inguinal and perineal areas (Kloos & Musselwhite, 1975; Kloos & Schleifer, 1975a; Kloos, 1986a). The proportion of *S. hominis* in the *Staphylococcus* community is often higher on the glabrous skin of the arms and legs than on other body regions. Strains of *S. hominis* colonize the skin of a person for relatively short periods of time, usually several weeks to several months, compared to many of the strains of the predominant species *Staphylococcus epidermidis* that persist for one to several years (Kloos, 1986b; Kloos *et al.*, 1992). *S. hominis* has occasionally been isolated from human

Abbreviation: HA, hydroxyapatite.

Table 1. List of *Staphylococcus hominis* strains and their source and year of isolation

Subspecies	Strain designation	Human source and residence (US state or Canada)	Year of isolation
S. hominis subsp. *hominis*	ATCC 27844^T	Skin (NC)	1971
S. hominis subsp. *hominis*	ATCC 27845	Skin (NC)	1972
S. hominis subsp. *hominis*	ATCC 27846	Skin (NC)	1971
S. hominis subsp. *hominis*	ATCC 27847	Skin (NC)	1971
S. hominis subsp. *hominis*	KH211	Skin (NC)	1972
S. hominis subsp. *hominis*	SM67	Skin (NC)	1971
S. hominis subsp. *hominis*	JL248	Skin (NC)	1972
S. hominis subsp. *hominis*	MCS 13	Skin (NJ)	1971
S. hominis subsp. *hominis*	GS16	Skin (NJ)	1973
S. hominis subsp. *hominis*	LK8152	Skin (NC)	1981
S. hominis subsp. *hominis*	LK8157	Skin (NC)	1981
S. hominis subsp. *hominis*	MY8418	Skin (NC)	1984
S. hominis subsp. *hominis*	MY8422	Skin (NC)	1984
S. hominis subsp. *hominis*	MY8429	Skin (NC)	1984
S. hominis subsp. *hominis*	RMS8423	Skin (NC)	1984
S. hominis subsp. *hominis*	DEM8518	Skin (NC)	1985
S. hominis subsp. *hominis*	NRC8511	Skin (NC)	1985
S. hominis subsp. *hominis*	NRC8517	Skin (NC)	1985
S. hominis subsp. *hominis*	NRC8519	Skin (NC)	1985
S. hominis subsp. *hominis*	NRC8520	Skin (NC)	1985
S. hominis subsp. *hominis*	NRC8528	Skin (NC)	1985
S. hominis subsp. *hominis*	DLB8510	Skin (NC)	1985
S. hominis subsp. *hominis*	ADW8535	Skin (NC)	1985
S. hominis subsp. *hominis*	MK8721	Skin (NC)	1987
S. hominis subsp. *hominis*	MK8742	Skin (NC)	1987
S. hominis subsp. *hominis*	MR8724	Skin (NC)	1987
S. hominis subsp. *hominis*	MR8726	Skin (NC)	1987
S. hominis subsp. *hominis*	MR8738	Skin (NC)	1987
S. hominis subsp. *hominis*	MS8	Unknown (NB, Canada)	1992
S. hominis subsp. *hominis*	MS9	Unknown	Unknown
S. hominis subsp. *hominis*	MS16	Unknown	Unknown
S. hominis subsp. *hominis*	MS25	Unknown	Unknown
S. hominis subsp. *hominis*	MS30	Unknown	Unknown
S. hominis subsp. *hominis*	D320	Blood (NC)	1992–1993
S. hominis subsp. *hominis*	D339	Blood (NC)	1992–1993
S. hominis subsp. *hominis*	D345	Blood (NC)	1992–1993
S. hominis subsp. *hominis*	R215	Blood (NJ)	1992–1993
S. hominis subsp. *novobiosepticus*	ATCC 700236^T	Blood (NJ)	1992–1993
S. hominis subsp. *novobiosepticus*	ATCC 700237	Unknown (IL)	1991
S. hominis subsp. *novobiosepticus*	ATCC 700238	Blood (NC)	1992–1993
S. hominis subsp. *novobiosepticus*	TB3328-1	Wound (NC)	1989
S. hominis subsp. *novobiosepticus*	R21	Blood (NJ)	1992–1993
S. hominis subsp. *novobiosepticus*	R41	Blood (NJ)	1992–1993
S. hominis subsp. *novobiosepticus*	R43	Blood (NJ)	1992–1993
S. hominis subsp. *novobiosepticus*	R72	Blood (NJ)	1992–1993
S. hominis subsp. *novobiosepticus*	R75	Blood (NJ)	1992–1993
S. hominis subsp. *novobiosepticus*	R90	Blood (NJ)	1992–1993
S. hominis subsp. *novobiosepticus*	R93	Blood (NJ)	1992–1993
S. hominis subsp. *novobiosepticus*	R201	Blood (NJ)	1992–1993
S. hominis subsp. *novobiosepticus*	R211	Blood (NJ)	1992–1993
S. hominis subsp. *novobiosepticus*	D2	Blood (NC)	1992–1993
S. hominis subsp. *novobiosepticus*	D17	Blood (NC)	1992–1993

Table 1. (*cont.*)

Subspecies	Strain designation	Human source and residence (US state or Canada)	Year of isolation
S. hominis subsp. *novobiosepticus*	D35	Blood (NC)	1992–1993
S. hominis subsp. *novobiosepticus*	D53	Blood (NC)	1992–1993
S. hominis subsp. *novobiosepticus*	D79	Blood (NC)	1992–1993
S. hominis subsp. *novobiosepticus*	D331	Blood (NC)	1992–1993
S. hominis subsp. *novobiosepticus*	MRL13378	Catheter tip (TN)	Unknown
S. hominis subsp. *novobiosepticus*	T/S2660	Unknown (CA)	1994
S. hominis subsp. *novobiosepticus*	T/S2662	Unknown (CA)	1994
S. hominis subsp. *novobiosepticus*	T/S3001	Blood (OK)	1994
S. hominis subsp. *novobiosepticus*	GRLD	Breast abcess (TX)	1996
S. hominis subsp. *novobiosepticus*	PKVW2	Blood (CO)	1996
S. hominis subsp. *novobiosepticus*	LKSD	Blood (IL)	1996
S. hominis subsp. 3	R241	Blood (NJ)	1992–1993
S. hominis subsp. 3	MS10	Unknown	Unknown
S. hominis subsp. 3	D90	Blood (NC)	1992–1993
S. hominis subsp. 3	D309	Blood (NC)	1992–1993

infections, but has been generally regarded as a contaminant or low-grade opportunistic pathogen of immunocompromised patients (Nord *et al.*, 1976; Sewell *et al.*, 1982; Bowman & Buck, 1984; Fleurette *et al.*, 1987; Kleeman *et al.*, 1993; Kloos & Bannerman, 1994). This species has been frequently isolated from blood cultures, although it has been considered to be clinically significant in only a small proportion of cases (Bowman & Buck, 1984; Pulverer, 1985; Ponce de Leon *et al.*, 1986; Kleeman *et al.*, 1993). During the past six years, an increasing proportion of blood culture isolates of *S. hominis* have been identified as aberrant strains of this species or misidentified as *Staphylococcus equorum* (Tural *et al.*, 1995; Weinstein *et al.*, 1995). This report is a description of these organisms based on a variety of phenotypic characters, antibiotic susceptibilities, partial sequence of the resident *mecA* gene, plasmid profiles, macrorestriction patterns of *Sma*I-digested chromosome fragments and DNA–DNA liquid hybridization. Although this specific group appears to have been formed from a relatively recent clone, it proved to be a taxonomically distinct subpopulation that has diverged significantly from the previously recognized *S. hominis* and, hence, should be placed into a separate subspecies. It has been given the name *S. hominis* subsp. *novobiosepticus* subsp. nov. In accordance with Rules 40a and 40b of the Bacteriological Code (Lapage *et al.*, 1975), the subpopulation previously recognized as *S. hominis* Kloos and Schleifer 1975, 68^{AL} (Kloos & Schleifer, 1975a) is named *S. hominis* subsp. *hominis* subsp. nov.

METHODS

Bacterial strains. The strains selected for the description of *S. hominis* subspecies, including their designations and sources, are listed in Table 1. The following species and strains of methicillin-resistant staphylococci were used for making *mecA* gene sequence comparisons with the *mecA* gene of the type strain ATCC 700236^T of *S. hominis* subsp. *novobiosepticus*: *Staphylococcus aureus* BB270 (Ryffel *et al.*, 1990); *S. aureus* TB4416; *S. epidermidis* WT55; *Staphylococcus sciuri* subsp. *sciuri* ATCC 29062^T; *S. sciuri* subsp. *sciuri* HE1L13; *S. sciuri* subsp. *sciuri* TT12b3; *S. sciuri* subsp. *carnaticus* ATCC 700058^T; *S. sciuri* subsp. *carnaticus* ATCC 700060; *S. sciuri* subsp. *carnaticus* ATCC 700059; *S. sciuri* subsp. *rodentium* ATCC 700061^T; *S. sciuri* subsp. *rodentium* ATCC 700063; *S. sciuri* subsp. *rodentium* ATCC 700062; *S. sciuri* subsp. *rodentium* Svv6; and *S. sciuri* subsp. *rodentium* MM16.

Phenotypic characterization. The following characteristics were determined as described previously (Kloos & Schleifer, 1975b; Schleifer & Kloos, 1975; Kloos *et al.*, 1976, 1991; Kloos & Bannerman) 1995): Gram-stained cell morphology and cell arrangements; colony morphology and pigmentation; motility; anaerobic growth in thioglycollate semi-solid medium; catalase activity; acetylmethylcarbinol (acetoin) production; nitrate reduction; oxidase activity; pyrrolidonyl arylamidase activity; aesculin hydrolysis; ornithine decarboxylase activity; urease activity; staphylocoagulase activity; lysostaphin susceptibility; haemolysis of sheep and bovine blood; and carbohydrate reactions. The presence of clumping factor and/or protein A was tested using the Staph Latex kit (Remel). Alkaline phosphatase, urease, β-glucosidase, β-glucuronidase and β-galactosidase activities and arginine utilization were tested with the API STAPH-IDENT system (bioMérieux Vitek) (Kloos & Wolfshohl, 1982). These same biochemical characteristics and additional biochemical profile data were also obtained with the MicroScan Dried Overnight (Conventional) Gram-Positive ID and MicroScan Rapid Gram-Positive ID panel systems (Dade MicroScan) (Kloos & George, 1991; Weinstein *et al.*, 1995). Colony size, pigmentation, lustre, profile and consistency were determined on freshly prepared P agar (Kloos *et al.*, 1991), tryptic soy agar (TSA; Difco), and TSA plus 5% sheep blood plates, following point-inoculation, incubation for 72 h at 35 °C, and storage at room temperature for an additional 2 d (Kloos & Schleifer, 1975a; Kloos & Bannerman, 1995). Biofilm and capsular

polysaccharide/adhesin were determined by methods previously described (Muller *et al*., 1993; Huebner *et al*., 1994).

Antibiotic susceptibilities were determined by disk-diffusion and broth-dilution (MIC) testing (Woods & Washington, 1995; National Committee for Clinical Laboratory Standards, 1997). Disk-diffusion testing involved the incubation of cultures on Mueller–Hinton agar plates at 35 °C for 24 h in the presence of various antibiotic disks, followed by the measurement of zone diameters of inhibition. However, novobiocin disk-diffusion was performed on both Mueller–Hinton agar and TSA plus 5% sheep blood plates. Novobiocin MICs were determined by broth macrodilution procedures using 1 ml Mueller–Hinton II broth (cation-adjusted) tubes and incubation of cultures at 35 °C for 24 h. The MICs of other antibiotics were determined by the automated MicroScan WalkAway MIC system using conventional Gram-Positive panels and by frozen broth microdilution panels prepared to National Committee for Clinical Laboratory Standards specifications (National Committee for Clinical Laboratory Standards, 1997).

Genomic DNA–DNA hybridization. Unlabelled and [*methyl*-^{3}H]thymidine-labelled total DNAs were isolated and purified using a modification of the procedures of Brenner *et al*. (1969) for use with staphylococcal species that are somewhat difficult to lyse (Kloos & Wolfshohl, 1979; Kloos *et al*., 1997). DNA–DNA reassociation reactions were performed at stringent (70 °C) and optimal (55 °C) conditions (Kloos & Wolfshohl, 1979; Kloos, 1980, 1998). At the termination of reactions, double-stranded (hybridized) DNA was separated from single-stranded (unreacted) DNA on hydroxyapatite (HA) by the batch procedure of Brenner *et al*. (1969). The relative binding (or DNA relatedness) was determined by normalizing the amount of DNA bound to HA in heterologous reactions to that bound in the homologous reaction, where labelled and unlabelled DNA are from the same organism.

PFGE of chromosome fragments. DNA was isolated and DNA-imbedded agarose plugs were prepared according to procedures described previously (George & Kloos, 1994; Kloos *et al*., 1998). Digestion of total DNA by the restriction endonuclease *Sma*I was performed in a 1·5 ml microcentrifuge tube by using a 125 µl assay conducted according to the instructions of the manufacturer (Gibco-BRL Life Technologies). A section ($2 \times 4 \times 1{\cdot}5$ mm) of the agarose plug prepared above was added to the assay tube and then incubated at 25 °C for 2 h with shaking at 150 r.p.m. PFGE procedures, macrorestriction pattern analysis and genome size determinations were conducted as previously described (George & Kloos, 1994). PFGE separation and size determination of the large *Sma*I-digest fragments (> 500 kb) were made on agarose gels run with a pulse of 60 s at 200 V for 15 h followed by a pulse of 90 s at 200 V for 10 h (results not shown). The Yeast Chromosome PFG Marker (New England BioLabs) was used to determine the size of the large *Sma*I-digest fragments.

Plasmid profile analysis. Procedures for plasmid isolation, agarose gel electrophoresis and determination of plasmid composition have been described previously (Kloos *et al*., 1981; DeGuglielmo *et al*., 1991). The Supercoiled DNA Ladder (Gibco-BRL Life Technologies), composed of 11 different supercoiled DNAs of 2067–16 210 bp, was used to estimate the size of plasmids.

DNA base composition. The DNA G+C content was determined by the thermal denaturation method of Marmur & Doty (1962) using a Guilford Response spectrophotometer with Response II thermal programming (Guilford Systems; Ciba Corning Diagnostics).

Detection of the *mecA* gene by PCR. Three sets of primers were used to detect overlapping regions of the *mecA* gene. The oligonucleotide primers chosen were based on published sequences of the *S. aureus* and *S. epidermidis mecA* gene (Ryffel *et al*., 1990). The first set of primers comprised 5′ dGATAACACCTTCTACACCTC 3′ (nt 33–52) and 5′ dCGATGCCTATCTCATATGCTG 3′ (nt 640–661), which gives an amplification product containing the promoter region and the first 499 bp of the coding frame. The second set of primers comprised 5′ dGGGATCATAGCGTCATTATTC 3′ (nt 520–540) and 5′ dCCTAATTCGAGTGCTACTCTAG 3′ (nt 1551–1572), which gives an amplification product of 1001 bp that is within the coding frame. The third set of primers comprised 5′ dTCTTGGGGTGGTTACAACG 3′ (nt 1457–1475) and 5′ dGAAGTTGTAGCAGGAACAC 3′ (nt 2392–2410), which gives an amplification product containing the last 680 bp of the coding frame plus 236 bp of the termination region.

Each strain to be tested for the presence of *mecA* was inoculated onto a 4 cm^2 area of a TSA plate containing 1% glycine and then incubated for 18 h at 35 °C. The culture was then scraped from the agar surface with a plastic loop and dispersed into 1 ml sterile distilled water contained in a 1·5 ml microcentrifuge tube. The cell suspension was centrifuged for 3 min in a Fisher Scientific model 235C microcentrifuge. Genomic DNA was isolated from the cell pellet using a QIAamp Tissue kit (QIAGEN) and instructions from the QIAamp Blood kit and QIAamp Tissue kit handbook, but with the addition of lysostaphin (550 µg ml^{-1}), lysozyme (1·39 mg ml^{-1}) and ribonuclease A (1 mg ml^{-1}; Sigma) to the incubation buffer and extending the incubation period from 30 min to 2–3 h. Additional modifications were made following the addition of proteinase K and the buffer AL (chaotropic salt solution; QIAGEN) to the lysed cell suspension by lowering the recommended incubation temperature of 70 °C to 55 °C and shortening the subsequent incubation time at 95 °C from 30 min to 5 min. Each PCR reaction was performed with 5 µl purified genomic DNA (approx. 500–700 ng) and the Expand High Fidelity PCR system (Boehringer Mannheim) in a total volume of 100 µl, according to the manufacturer's instructions. Primers were annealed at 55 °C for 15 s in the first series of PCR reactions. All negative reactions were repeated with an annealing temperature of 42 °C to detect other closely related *mecA* gene homologues. Amplification products were purified using the Promega Wizard PCR Preps DNA Purification system according to the manufacturer's instructions. The purified products were sequenced at the DNA Sequencing Facility, Iowa State University, Ames, IA, USA. In addition, purified products were digested with *Cla*I and the resulting fragments were separated by agarose gel electrophoresis according to previously described procedures (George & Kloos, 1994).

RESULTS AND DISCUSSION

DNA–DNA hybridization and DNA base composition

The DNA relationships and DNA G+C content of selected strains of *S. hominis* subsp. *hominis* and *S. hominis* subsp. *novobiosepticus* and a third divergent population of *S. hominis*, provisionally designated subsp. 3, are shown in Table 2. As can be seen from the data, each subspecies forms a separate DNA similarity

Table 2. Results of hybridization of staphylococcal DNAs with [*methyl*-^{3}H]thymidine-labelled DNAs from each of the *S. hominis* subspecies

Species and subspecies	Strain	DNA G+C content (mol%)	Relative binding (%) at 70 °C with labelled DNA from:		
			S. hominis subsp. *hominis* (ATCC 27844^T)	*S. hominis* subsp. *novobiosepticus* (ATCC 700236^T)	*S. hominis* subsp. 3 (MS10)
S. hominis subsp. *hominis*	ATCC 27844^T	34	**100**	70	78
	ATCC 27847	30	**88**	73	81
	MS9		**94**	63	75
	D339		**93**	68	78
	D345	34	**84**	77	77
S. hominis subsp. *novobiosepticus*	ATCC 700236^T	35	62	**100**	85
	R90		62	**93**	83
	R75		74	**100**	85
	R211		75	**94**	77
	ATCC 700237	35	73	**98**	83
	D314		72	**82**	83
	ATCC 700238		76	**95**	85
	R201		75	**88**	82
S. hominis subsp. 3	MS10	33	70	64	**100**
	D90		70	77	**89**
	D309		70	76	**83**
	R241		65	69	**92**

group at the stringent (70 °C) criterion. Strains of the same subspecies demonstrated a high level of DNA relatedness (mean relative binding of 91 ± 5%), whereas strains of different *S. hominis* subspecies demonstrated a lower level of DNA relatedness (mean relative binding of 74 ± 7%). The different *S. hominis* subspecies could not be clearly distinguished at the optimal (55 °C) criterion (mean relative binding of 90 ± 5%) (data not shown). The DNA G+C content of selected strains of the subspecies of *S. hominis* was 30–35 mol%.

Macrorestriction pattern analysis and estimation of genome size

The restriction endonuclease *Sma*I (recognition site 5′ CCCGGG 3′), an enzyme commonly used to digest the chromosomes of staphylococci for macrorestriction pattern analysis and for distinguishing their subspecies (Lina *et al*., 1992; George & Kloos, 1994; Bannerman *et al*., 1995), cleaved the *S. hominis* chromosome into 8–12 fragments, depending upon the particular strain. Most of the digest fragments were well-separated following PFGE (Fig. 1). The smallest *Sma*I-digest fragments of sizes 2, 3, 44 and 78 kb were present in all of the 46 *S. hominis* strains examined by PFGE. This pattern has not been observed with other *Staphylococcus* species. As can be seen in the figures, *S. hominis* subsp. *novobiosepticus* produced a different fragment pattern from that of *S. hominis* subsp. *hominis* and *S. hominis* subsp. 3. *Sma*I-digest fragments of sizes 185, 254 and about 950 kb are highly conserved among the *S. hominis* subsp. *novobiosepticus* strains. Several other fragments of sizes 210–228 and 680–710 kb are also usually present in this subspecies. Furthermore, most strains (68%) of *S. hominis* subsp. *novobiosepticus* failed to exhibit a 110 kb fragment that is usually present in the other subspecies. With the exception of the species-specific fragments mentioned above and the conserved fragment of 110 kb, most of the *Sma*I-digest fragments of the chromosome of *S. hominis* subsp. *hominis* are polymorphic. Likewise, most of the *Sma*I-digest fragments of the chromosome of *S. hominis* subsp. 3 are polymorphic. Due to the small number of strains available and the inability of the phenotypic characterization (data not shown) to distinguish *S. hominis* subsp. 3 from *S. hominis* subsp. *hominis*, this subspecies will not be formally introduced at this time.

The estimated size of the *S. hominis* subsp. *hominis* chromosome is 2265 ± 81 kb, whereas the size of the *S. hominis* subsp. *novobiosepticus* chromosome is estimated to be 2453 ± 84 kb, which is significantly larger.

Phenotypic characterization

Cells of the *S. hominis* subspecies were Gram-positive cocci (1·0–1·5 μm in diameter), non-motile and non-spore-forming; they were arranged mostly as tetrads and occasionally as pairs. Although the subspecies are facultative anaerobes, growth was much better under aerobic conditions. They failed to grow in the anaerobic portion of the thioglycollate semi-solid medium within 48 h, but some strains grew weakly in the anaerobic portion by 3–5 d. All strains were positive for catalase and negative for oxidase (Microdase disk) activities, resistant to the 0·04 U bacitracin (Taxo A) disk and lysozyme (25 μg ml^{-1}), slightly susceptible to lysostaphin (200 μg ml^{-1}), and susceptible to the 100 μg furazolidone disk. All strains were positive for urease activity (with the exception of 10 of the 784 strains of *S. hominis* subsp. *hominis*) and produced acid aerobically from D-glucose, *β*-D-fructose, maltose, sucrose (often slowly), and glycerol. A small proportion (12%) of the *S. hominis* subsp. *hominis* strains produced ammonia from arginine, whereas none of the *S. hominis* subsp. *novobiosepticus* strains utilized arginine. All strains were negative for staphylocoagulase, latex agglutination, ornithine decarboxylase, alkaline

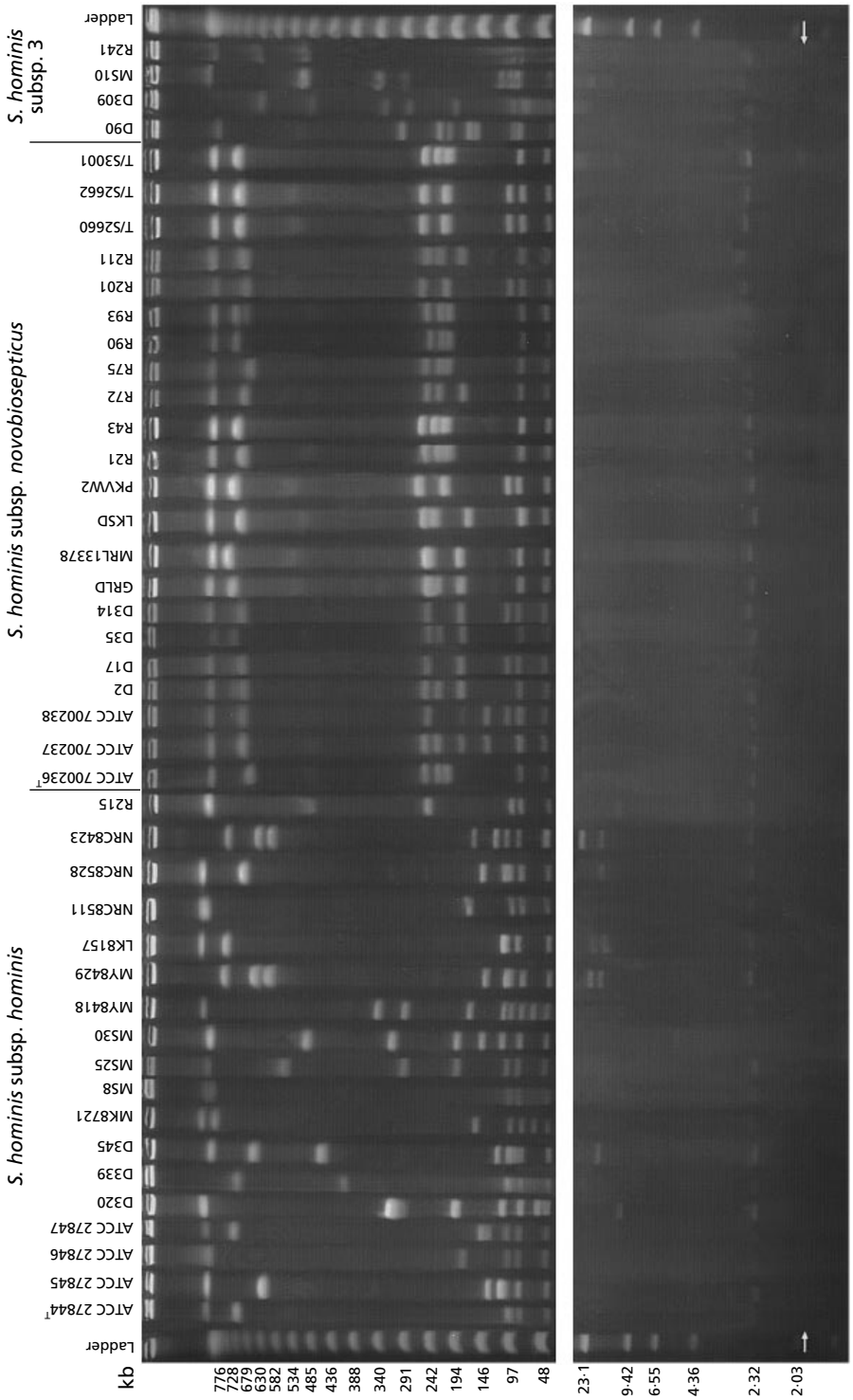

Fig. 1. *Sma*I macrorestriction patterns of the chromosome of *S. hominis* subspecies. The upper panel is a composite photograph of *Sma*I-digest fragments ($\geq$ 44 kb) separated by PFGE on agarose gels run with a ramped pulse of 15–55 s at 200 V for 22 h. The lower panel is a composite photograph of small *Sma*I-digest fragments ($<$ 44 kb) separated by PFGE on agarose gels run with a ramped pulse of 1–12 s at 150 V for 16 h. The very small 2 kb *Sma*I-digest fragments are difficult to detect in the photograph and their position on the gel is indicated by the arrows. Ladder lanes contain molecular size markers (see Methods).

Table 3. Variable characteristics and antibiotic susceptibilities of *S. hominis* subspecies

Characteristic	Positive or resistant (and weak positive or intermediate) strains (%)		
	S. hominis subsp. *hominis*		*S. hominis* subsp. *novobiosepticus*
	Normal skin isolates ($n = 712$)	Clinical isolates ($n = 72$)	Clinical isolates ($n = 26$)
Colony diameter $\geqslant$ 5 mm	65	68	100
Colony pigmentation	50	10	0
Acetylmethylcarbinol (acetoin)	63	42	27
Acid (aerobic) produced from:			
D-Trehalose	84	68	0
N-Acetyl-D-glucosamine	86	83 (3)	0
α-Lactose	41 (14)	64 (10)	69 (19)
D-Melezitose	60 (10)	42 (22)	23 (19)
D-Mannitol	13	14	0
Resistance to:*			
Novobiocin (5 μg)	0·6	0	100
Nalidixic acid (30 μg)	ND	27	100
Penicillin G (10 U)	43	75	100
Oxacillin (1 μg)	4	14 (3)	100
Methicillin (5 μg)	3 (1)	11 (6)	77 (23)
Gentamicin (10 μg)	2†	14	96 (4)
Streptomycin (10 μg)	3	10	100
Erythromycin (15 μg)	18	44	88
Clindamycin (2 μg)	10	27	88
Chloramphenicol (30 μg)	5	6	65
T/S (1·25/23·75 μg)	7†	19	58 (38)
Ciprofloxacin (5 μg)	0†	8	54 (8)
Tetracycline (30 μg)	35	47	19

ND, Not determined.

* Interpretation of zone of inhibition diameter for antibiotic disks: novobiocin-resistant, 10–15 mm (Mueller–Hinton Agar) or 0–11 mm (TSA with 5% sheep blood); nalidixic acid-resistant, none; penicillin G-resistant, 0–18 mm; oxacillin-, gentamicin-, clindamycin-, chloramphenicol-, trimethoprim/sulfamethoxazole- (T/S) or tetracycline-resistant, 0–10 mm; oxacillin intermediate, 11–12 mm; gentamicin intermediate, 13–14 mm; T/S intermediate, 11–15 mm; methicillin-resistant, 0–9 mm; methicillin intermediate, 10–13 mm; streptomycin-resistant, none; erythromycin-resistant, 0–12 mm; ciprofloxacin-resistant, 0–15 mm; ciprofloxacin intermediate, 16–20 mm.

† Number of strains tested was 90.

phosphatase (with the exception of two *S. hominis* subsp. *hominis* strains), pyrrolidonyl arylamidase (with the exception of three *S. hominis* subsp. *hominis* strains), β-glucosidase, β-glucuronidase and β-galactosidase activities, and failed to produce acid aerobically from D-mannose (with the exception of six *S. hominis* subsp. *hominis* strains), D-xylose, L-arabinose, D-sorbitol, D-cellobiose, salicin and D-raffinose. Characteristics that are variable for one or both *S. hominis* subspecies are listed in Table 3. Several of the phenotypic characters were expressed differently among the subspecies and therefore had taxonomic value. For example, none of the strains of *S. hominis* subsp. *novobiosepticus* produced acid aerobically from D-trehalose and *N*-acetyl-D-glucosamine, whereas 68–86% of the strains of *S. hominis* subsp. *hominis* produced acid aerobically from D-trehalose and 86% of the strains of this subspecies produced acid aerobically from *N*-acetyl-D-glucosamine. Also, none of the colonies of *S. hominis* subsp. *novobiosepticus* was pigmented. None of the five selected strains (including the type strains) of each subspecies produced capsular polysaccharide/adhesin. These same strains produced only very low amounts of biofilm and the two subspecies could not be distinguished on the basis of biofilm production.

Compared to the patterns of antibiotic susceptibilities observed with the other staphylococcal species and subspecies that are members of the *S. epidermidis*

species group (including *S. epidermidis*, *Staphylococcus capitis* subsp. *capitis*, *S. capitis* subsp. *ureolyticus*, *Staphylococcus caprae*, *S. hominis* subsp. *hominis*, *Staphylococcus haemolyticus*, *Staphylococcus warneri*, *Staphylococcus pasteuri* and *Staphylococcus saccharolyticus*) (Kloos, 1997, 1998), *S. hominis* subsp. *novobiosepticus* is most unusual in that all of the strains tested, using the breakpoints established for *Staphylococcus saprophyticus* (Kloos & Bannerman, 1995), were resistant to novobiocin and, using the breakpoints established for *S. aureus* (Woods & Washington, 1995), were resistant to penicillin G, oxacillin and streptomycin, and were either resistant or had intermediate resistance to methicillin, kanamycin and gentamicin. Furthermore, 88% of the strains of this subspecies were resistant to erythromycin and clindamycin, 65% of strains were resistant to chloramphenicol, and 96 and 62% of strains were either resistant or had intermediate resistance to trimethoprim/sulfamethoxazole and ciprofloxacin, respectively. All strains were susceptible to vancomycin. With *S. hominis* subsp. *novobiosepticus*, the disk zone of inhibition diameter for novobiocin was significantly less when this antibiotic was tested on TSA plus 5% sheep blood agar (8 ± 1 mm) than when it was tested on Mueller–Hinton agar (13 ± 2 mm).

Up until the discovery of this subspecies, novobiocin resistance was not considered to be a normal property of members of the *S. epidermidis* species group, but rather was recognized as an intrinsic property of members of the *S. saprophyticus* species group, and its peripheral species, and the *S. sciuri* species group (Kloos *et al.*, 1991). All strains of *S. hominis* subsp. *novobiosepticus* were resistant to nalidixic acid, which, like novobiocin, is a DNA gyrase inhibitor. Although breakpoints have not been established for nalidixic acid in staphylococci, resistance may be inferred by the absence of growth inhibition with the 5 μg disk and no inhibition or only a small inhibition diameter of 8–10 mm with the 30 μg disk. The range of novobiocin MICs among *S. hominis* subsp. *novobiosepticus* strains was 4–16 μg ml^{-1}, which is comparable to the range noted for members of the *S. saprophyticus* and *S. sciuri* species groups (Schleifer, 1986). The novobiocin MICs for the novobiocin-susceptible *S. hominis* subsp. *hominis* strains were $\leqslant 0{\cdot}25$ μg ml^{-1}. The range of oxacillin MICs among *S. hominis* subsp. *novobiosepticus* strains was 16 to >64 μg ml^{-1}, with the exception of strains D35 and MRL13378, which had an MIC of 0·25 μg ml^{-1}. Although these exceptional strains were classified as susceptible to oxacillin by microdilution testing, they appeared to be resistant by disk-diffusion testing, exhibiting a zone of inhibition diameter of 10 mm with the 1 μg oxacillin disk. However, strains having the higher oxacillin MICs also exhibited a higher level of resistance by disk-diffusion testing, as indicated by the absence of growth inhibition, suggesting that there is some variation in the level of oxacillin resistance among strains of this subspecies.

Plasmid profiles

The plasmid profiles of *S. hominis* subsp. *novobiosepticus* strains were rather similar, though some differed by the presence or absence of one or two plasmids (Fig. 2). It is suspected that the similarity is due, in part, to the presence of common antibiotic-resistance plasmids responsible for producing a portion of the resistance pattern observed in antibiograms. For example, all of the 17 erythromycin- and clindamycin-resistant strains (of a total of 20 *S. hominis* subsp. *novobiosepticus* strains examined) carried a plasmid of about 2·2–2·4 kb, demonstrating several conformational forms, all 13 chloramphenicol-resistant strains carried a plasmid of about 3·8 kb, and the four tetracycline-resistant strains carried a plasmid of about 4·4 kb (Kloos, 1998). Fourteen (70%) strains carried a small cryptic plasmid of about 1 kb, which demonstrated several conformational forms and all strains, with the possible exception of strain R43, carried relatively large plasmids of >16 kb. However, the larger plasmids were not examined further for either their size similarities or relationships to antibiotic resistance.

Plasmid profiles of *S. hominis* subsp. *hominis*, as shown in Fig. 2 and in a prior publication (Kloos *et al.*, 1981), are more diverse, demonstrating a wide range of different cryptic plasmids and generally fewer of the common antibiotic resistance plasmids suspected of being present in *S. hominis* subsp. *novobiosepticus*.

Identification of the *mecA* gene homologue in *S. hominis* subsp. *novobiosepticus*

Each of the three sets of primers chosen to detect overlapping regions of the *mecA* gene present in the methicillin-resistant *S. aureus* (MRSA) strain BB270 and *S. epidermidis* (MRSE) strain WT55 (Ryffel *et al.*, 1990) produced a large amount (>1 μg μl^{-1}) of amplification product of the predicted size from genomic DNA of the selected oxacillin- and methicillin-resistant strains *S. aureus* TB4416, *S. sciuri* subsp. *rodentium* MM16, *S. sciuri* subsp. *rodentium* ATCC 700063, *S. hominis* subsp. *hominis* ATCC 27836, *S. hominis* subsp. *novobiosepticus* ATCC 700236^{T} and *S. hominis* subsp. *novobiosepticus* ATCC 200237 when PCR reactions were performed at the annealing temperature of 55 °C. These same primers either failed to yield detectable amounts or produced only very small amounts (<20 ng μl^{-1}) of amplification product of the predicted size from genomic DNA of the borderline methicillin-resistant/susceptible strains *S. sciuri* subsp. *rodentium* ATCC 700061^{T}, *S. sciuri* subsp. *rodentium* ATCC 700062, *S. sciuri* subsp. *rodentium* Svv6, *S. sciuri* subsp. *sciuri* ATCC 29062^{T}, *S. sciuri* subsp. *sciuri* HE1L13, *S. sciuri* subsp. *sciuri* TT12b3, *S. sciuri* subsp. *carnaticus* ATCC 700058^{T}, *S. sciuri* subsp. *carnaticus* ATCC 700060 and *S. sciuri* subsp. *carnaticus* ATCC 700059 at the annealing temperature of 55 °C. However, when PCR reactions were performed under conditions of lower stringency, i.e. at the annealing temperature of

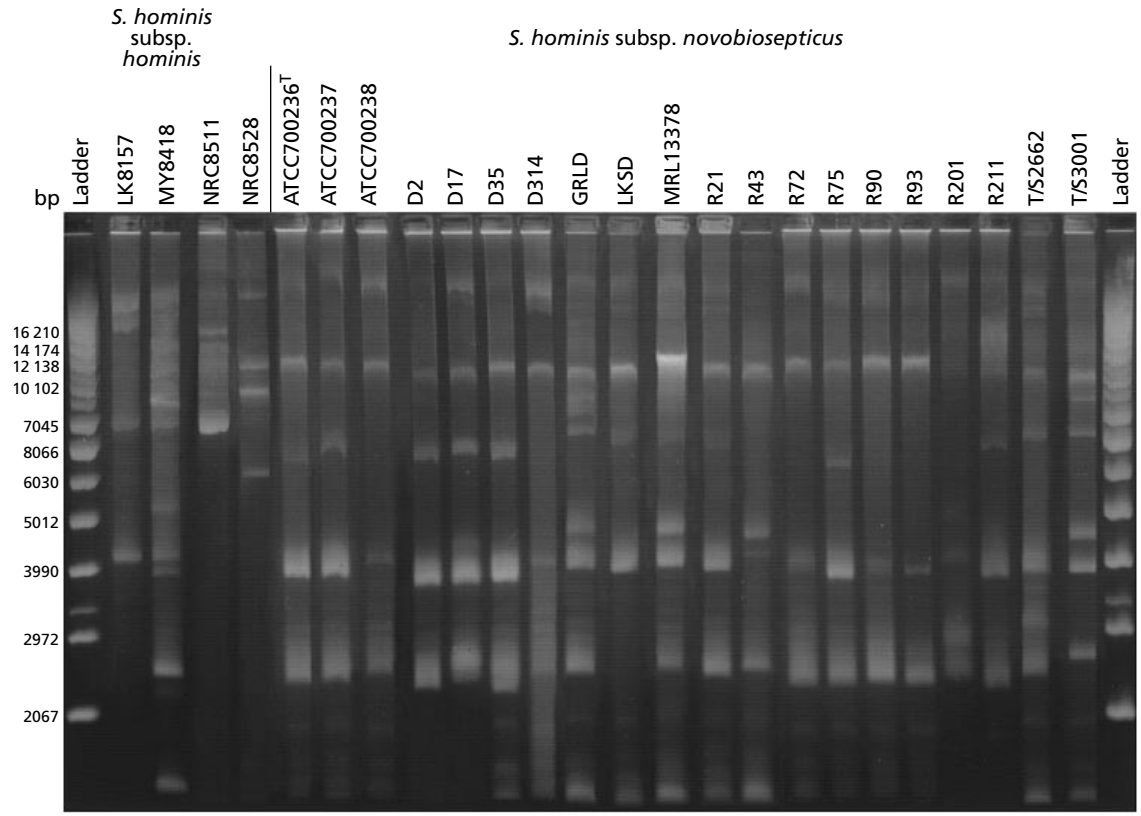

Fig. 2. Plasmid profiles of *S. hominis* subsp. *novobiosepticus* and several strains of *S. hominis* subsp. *hominis*. Ladder lanes contain molecular size markers (see Methods).

42 °C, all of the above strains exhibited large amounts (>1 μg μl^{-1}) of amplification product of the predicted size. It has been recently reported that this second group of *S. sciuri* strains has a *mecA* gene homologue that is different from that of the first group of methicillin-resistant *S. sciuri* subsp. *rodentium* and *S. aureus* strains on the basis of the number of *Cla*I sites present in each homologue and phenotypic characterization (Couto *et al.*, 1996; Kloos *et al.*, 1997). The present study suggests that the *mecA* homologue (referred to here as homologue A) of the first group of methicillin-resistant staphylococci is intrinsic to *S. hominis* subsp. *novobiosepticus*. The genomic DNA of all 17 *S. hominis* subsp. *novobiosepticus* strains tested with the second set of primers (giving an amplification product of 1001 bp that is within the coding frame of *mecA*) generated large amounts of the expected amplification product at the more stringent annealing temperature. The identity of homologue A was further verified by *Cla*I digestion and by sequencing of the amplification product generated by the second set of primers. The homologue A amplification product of the above strains contained one *Cla*I site at nt 1427–1432, whereas the homologue B amplification product of *S. sciuri* subsp. *sciuri*, *S. sciuri* subsp. *carnaticus* and certain strains of *S. sciuri* subsp. *rodentium* did not contain a *Cla*I site, with the exception of the amplification product of *S. sciuri* subsp. *sciuri* strain TT12b3, which contained *Cla*I sites at nt 1130–1135 and 1505–1510. A comparison of the 1001 bp amplification product sequences of reference strains of staphylococci and the type strain of *S. hominis* subsp. *novobiosepticus* is shown in Fig. 3. The sequences from homologue B share approximately 79–80 % base pair similarity to those of homologue A. The sequence of homologue A from the type strain of *S. hominis* subsp. *novobiosepticus* shares 99·9 % base pair similarity to the sequence of homologue A from the reference *S. epidermidis* strain WT55.

Description of *Staphylococcus hominis* Kloos and Schleifer 1975, 68^{AL} emend. Kloos *et al.* 1998

The description of *Staphylococcus hominis* is the same as that of the original description (Kloos & Schleifer, 1975a), except that it is amended to include strains that are either novobiocin-susceptible or novobiocin-resistant. *S. hominis* was originally described as a novobiocin-susceptible species.

Description of *Staphylococcus hominis* subsp. *hominis* subsp. nov.

The description of *S. hominis* subsp. *hominis* is based on the characteristics of 240 strains of *S. hominis* sp. nov. (Kloos & Schleifer, 1975a) isolated from human skin in 1971–1973, 472 strains isolated from human

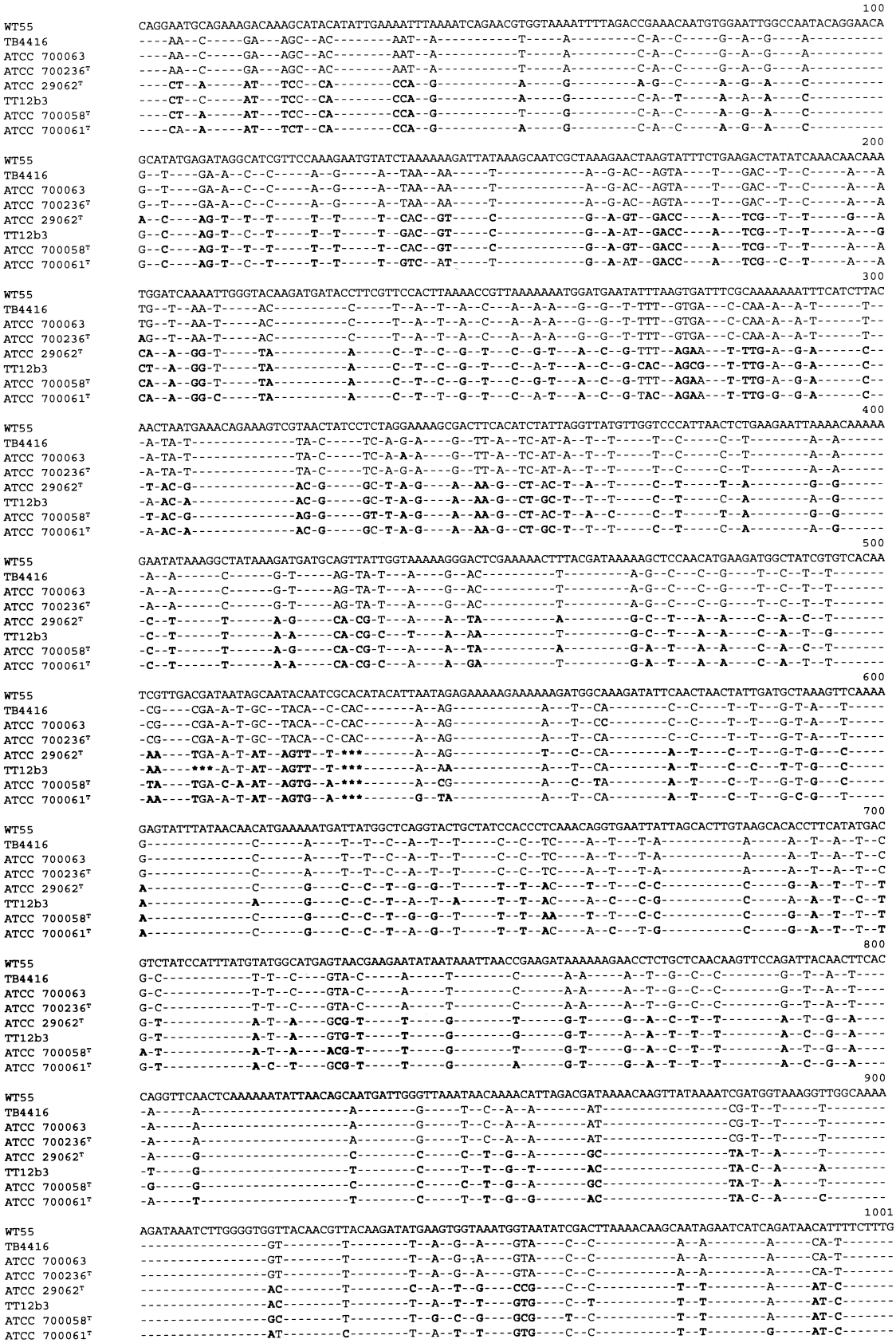

Fig. 3. For legend see facing page.

skin in 1974–1988, and 72 strains isolated from clinical specimens in 1989–1993. This subspecies is the type subspecies of *S. hominis* and contains the type strain according to Rules 40a and 40b of the Bacteriological Code (Lapage *et al.*, 1975).

Colonies are either pigmented (cream-yellow to yellow-orange) or unpigmented (grey-white), opaque, entire, butyrous, raised to very slightly umbonate, and grow to a diameter of 4–6 mm on P agar and TSA. Growth in the anaerobic portion of a semi-solid thioglycollate medium is negative within 48 h. Optimum growth temperature is 35 °C. Cultures grown on TSA supplemented with either sheep or bovine blood do not exhibit haemolysis. All strains produce acid aerobically from D-glucose, β-D-fructose, maltose, sucrose and glycerol. Acid is not produced from D-xylose, L-arabinose, D-sorbitol, D-cellobiose, salicin and D-raffinose. Most strains (83%) produce acid aerobically from D-trehalose and 86% produce acid from *N*-acetyl-D-glucosamine. Some strains (57%) produce weak to moderate amounts of acid aerobically from α-lactose and 69% of strains produce weak to moderate amounts of acid from D-melezitose. Only a small percentage of strains produce acid aerobically from D-mannitol (13%) and D-mannose (0·8%). Most strains (99%) are positive for urease activity and 12% of strains are positive for arginine utilization. Some strains (61%) produce acetoin from glucose metabolism. Capsular polysaccharide/adhesin is not produced. Strains may be either susceptible or resistant to penicillin, oxacillin, methicillin, kanamycin, gentamicin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol, trimethoprim/sulfamethoxazole and ciprofloxacin. Populations subjected to antibiotic pressure often exhibit multiple resistance. Other characteristics of *S. hominis* subsp. *hominis* that are uniformly positive or negative for the species are listed above in the section on phenotypic characterization and in the original description of *S. hominis*. The DNA G+C content is 30–36 mol%. *S. hominis* subsp. *hominis* is commonly isolated from human skin and occasionally from clinical specimens.

Description of the type strain. The type strain of *S. hominis* subsp. *hominis* is DM 122^T (= ATCC 27844^T), which was the type strain of *S. hominis* (Kloos & Schleifer, 1975a). It has all of the uniform characteristics of the subspecies described above, produces acid aerobically from *N*-acetyl-D-glucosamine, and has other characteristics as indicated in the original description.

Description of *Staphylococcus hominis* subsp. *novobiosepticus* subsp. nov.

Staphylococcus hominis subsp. *novobiosepticus* (novo.bio.sep′ti.cus. M.L. adj. combination of *novobio* pertaining to the property of novobiocin resistance and *septicus* pertaining to growth in blood).

The description below is based on the characteristics of 26 strains isolated in 1989–1996. Colonies are unpigmented (grey-white), opaque, entire, butyrous, slightly convex to umbonate, and grow to a diameter of 5·4 ± 0·3 mm on P agar and 5·8 ± 0·3 mm on TSA. Growth in the anaerobic portion of a semi-solid thioglycollate medium is negative within 48 h. Optimum growth temperature is 35 °C. Cultures grown on TSA supplemented with either sheep or bovine blood do not exhibit haemolysis. All strains produce acid aerobically from D-glucose, β-D-fructose, maltose, sucrose and glycerol. Acid is not produced from D-trehalose, *N*-acetyl-D-glucosamine, D-mannitol, D-mannose, D-xylose, L-arabinose, D-sorbitol, D-cellobiose, salicin and D-raffinose. Most strains (88%) produce weak to moderate amounts of acid aerobically from α-lactose and 42% of strains produce weak to moderate amounts of acid from D-melezitose. All strains are positive for urease activity and negative for arginine utilization. Some strains (27%) produce acetoin from glucose metabolism. Capsular polysaccharide/adhesin is not produced. All strains are resistant to novobiocin, nalidixic acid, penicillin, oxacillin and streptomycin, and are resistant or have intermediate resistance to methicillin, kanamycin and gentamicin. Until a much larger number of strains are tested for antibiotic susceptibilities, some caution should be taken concerning the identification of a specific antibiotic resistance as intrinsic to the subspecies, even though all strains tested in this study proved to be resistant to the antibiotic. Many strains are resistant to erythromycin, clindamycin, chloramphenicol, trimethoprim/sulfamethoxazole and ciprofloxacin. Other characteristics of *S. hominis* subsp. *novobiosepticus* that are uniformly positive or negative for the species are listed above in the section on phenotypic characterization. The DNA G+C content is 35 mol%. *S. hominis* subsp. *novobiosepticus* is commonly isolated from human blood cultures and occasionally from other clinical specimens. If this subspecies is a resident on human skin, it probably exists as very small populations of cells and requires enrichment, e.g. by additional growth in a suitable medium or body fluids, for detection. Although *S. hominis* subsp. *hominis* is commonly isolated from

Fig. 3. Comparison of the nucleotide sequences of the 1001 bp PCR amplification product of *mecA* from *S. hominis* subsp. *novobiosepticus* ATCC 700236^T and the reference strains *S. epidermidis* WT55, *S. aureus* TB4416, *S. sciuri* subsp. *sciuri* ATCC 29062^T and TT12b3, *S. sciuri* subsp. *carnaticus* ATCC 70058^T, and *S. sciuri* subsp. *rodentium* ATCC 700061^T and ATCC 700063. Dashes indicate residues identical to those of the sequence of *S. epidermidis* strain WT55 (Ryffel *et al.*, 1990), while the asterisks indicate gaps or deletions introduced to maximize alignment. Residues that are different from the sequence of strain WT55 are indicated by bold letters. The first four strains (WT55, TB4416, ATCC 700063 and ATCC 700236^T) listed in the alignment of sequences have the homologue A of *mecA*, whereas the second set of four strains (ATCC 29062^T, TT12b3, ATCC 700058^T and ATCC 700061^T) have the homologue B of *mecA*.

Table 4. Characteristics useful for differentiating *S. hominis* subspecies from one another and the closely related species *S. haemolyticus* and the occasionally confused species *S. saprophyticus* and *S. equorum*

+, 90% or more of the strains are positive; ±, 90% or more of the strains are weakly positive; d, 11–89% of the strains are positive (the percentage of positive strains is also given); −, 90% or more of the strains are negative. Parentheses indicate that a response is delayed.

Characteristic	*S. hominis* subsp. *hominis*	*S. hominis* subsp. *novobiosepticus*	*S. haemolyticus*	*S. saprophyticus*	*S. equorum*
Novobiocin resistance	−	+	−	+	+
Anaerobic growth	(±)	(±)	(+)	(+)	−
Urease	+	+	−	+	+
Pyrrolidonyl arylamidase	−	−	+	−	−
β-Glucuronidase	−	−	d 48	−	+
β-Galactosidase	−	−	−	+	d 50
Arginine utilization	d 12	−	+	−	−
Acid (aerobic) produced from:					
D-Trehalose	d 76	−	+	+	+
N-Acetyl-D-glucosamine	d 86	−	+	d 82	d 67
D-Mannitol	d 14	−	d 58	d 88	+
D-Mannose	−	−	−	−	+
D-Xylose	−	−	−	−	+
L-Arabinose	−	−	−	−	+

human skin and is considered to be a major species of the cutaneous ecosystem, there are no reports of the isolation of *S. hominis* subsp. *novobiosepticus* from human skin.

Description of the type strain. The type strain of *S. hominis* subsp. *novobiosepticus* is R22^T (= ATCC 700236^T); it was isolated from a human blood culture. It has all of the uniform characteristics of the subspecies described above and has the following properties listed below.

Spherical cells (diameter 1·2–1·5 μm) occur mostly in tetrads and sometimes in pairs. Circular colonies are 5·1–5·5 mm in diameter on P agar and 5·5–5·8 mm in diameter on TSA. They have an entire edge, slightly convex to umbonate profile, dull surface, butyrous consistency, and are opaque and unpigmented (grey-white). Facultatively anaerobic, delayed and weak growth occurs in the anaerobic portion of a thioglycollate medium. Acetoin is produced from glucose. Acid is produced aerobically from D-glucose, β-D-fructose, maltose, sucrose, glycerol and α-lactose, but not from D-melezitose, D-trehalose, D-mannitol, D-mannose, *N*-acetyl-D-glucosamine, D-xylose, L-arabinose, D-sorbitol, D-cellobiose, salicin and D-raffinose. Resistant to novobiocin, nalidixic acid, penicillin G, oxacillin, methicillin, kanamycin, gentamicin, streptomycin, chloramphenicol, erythromycin, clindamycin and ciprofloxacin, and susceptible to tetracycline, trimethoprim/sulfamethoxazole, and vancomycin. The DNA G+C content is 35 mol%.

Distinguishing characteristics. The phenotypic characteristics that are useful for distinguishing *S. hominis* subsp. *novobiosepticus* from *S. hominis* subsp. *hominis*, the closely related species *S. haemolyticus*, and the occasionally confused species *S. saprophyticus* and *S. equorum* are summarized in Table 4. *S. hominis* subsp. *novobiosepticus* can be distinguished from *S. hominis* subsp. *hominis* by the combined characteristics of novobiocin resistance and failure to produce acid aerobically from D-trehalose and *N*-acetyl-D-glucosamine. It can be differentiated from *S. haemolyticus* by the combined characteristics of novobiocin resistance, failure to produce acid aerobically from D-trehalose and *N*-acetyl-D-glucosamine, positive urease activity, and negative pyrrolidonyl arylamidase activity and arginine utilization. *S. saprophyticus* can be distinguished from both subspecies of *S. hominis* by its positive β-galactosidase activity and it can be distinguished from *S. hominis* subsp. *novobiosepticus*, in particular, on the basis of its ability to produce acid aerobically from D-trehalose. The animal species *S. equorum* can be distinguished from both subspecies of *S. hominis* on the basis of its ability to produce acid aerobically from D-xylose and L-arabinose and positive β-glucuronidase activity, and can be further distinguished from *S. hominis* subsp. *novobiosepticus* by its ability to produce acid aerobically from D-trehalose, D-mannitol and D-mannose.

The combined resistance to novobiocin (and nalidixic acid) and oxacillin (and methicillin) may be intrinsic to the subspecies *S. hominis* subsp. *novobiosepticus*. One attractive hypothesis concerning the origin of the combined resistance is that the genes controlling resistance to novobiocin (*nov* or *gyrB*) and methicillin (*mecA*) have been introduced simultaneously by the acquisition of heterologous DNA from a methicillin-

resistant strain of one of the novobiocin-resistant species that is a member of the *S. sciuri* or the *S. saprophyticus* species groups. The larger genome size of *S. hominis* subsp. *novobiosepticus* compared to *S. hominis* subsp. *hominis* may be, in part, a consequence of the acquisition of such heterologous DNA. In various strains of *S. aureus*, *S. epidermidis* and *S. haemolyticus*, it has been shown that the *nov* (or *gyrB*), *gyrA* and *mecA* genes are relatively closely linked on the chromosome and occur together on one of the *Sma*I fragments (Fey *et al*., 1997; Iandolo *et al*., 1997). An alternative hypothesis worth considering is that insertion of the *mecA* gene and its flanking sequences into the chromosome of *S. hominis* subsp. *novobiosepticus* might have an effect on the expression of a closely linked, resident *nov* gene, converting the host from novobiocin-susceptible to novobiocin-resistant. It would appear that the subspecies *S. hominis* subsp. *novobiosepticus* was formed relatively recently, based on the rather similar macrorestriction patterns of chromosomal DNA, colony morphology, plasmid profiles and antibiograms of different strains. This subspecies exhibits a comparatively low degree of polymorphism. The question remains as to when *S. hominis* subsp. *novobiosepticus* was formed. For example, was it formed prior to the acquisition of antibiotic-resistance genes now intrinsic to the subspecies or was it formed after or as a consequence of the acquisition of antibiotic-resistance genes and/or their flanking sequences that perhaps directed a new evolutionary path? One wonders how the path taken by this subspecies relates to the evolutionary progress of methicillin-resistant *S. aureus* (MRSA) strains which appear to be in the process of subspeciation.

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