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Drug-resistant coagulase-negative skin staphylococci

Evaluation of four marker systems and epidemiology in an orthopaedic ward

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SUMMARY

Drug-resistant coagulase-negative staphylococci (DRCNS) in orthopaedic patients and ward staff were studied. A significant increase in the DRCNS carriage rate was observed among the 16 patients studied after 14 days of hospitalization with levels approaching that of the staff. Patients receiving dicloxacillin prophylaxis ($n = 9$) were more likely to be colonized with methicillin-resistant CNS, while patients receiving no antibiotics ($n = 7$) became to a larger extent colonized with multiple DRCNS. The combined data from species determination, biochemical, plasmid, and antibiogram typing revealed a considerable diversity among DRCNS; 64 types were distinguished among 112 DRCNS isolates selected for study after exclusion of apparently duplicate isolates. Plasmid plus antibiogram typing yielded almost as many types (61); whereas species determination plus antibiogram distinguished only 33 types. Although a novel computerized 96-reaction boityping method alone enabled differentiation of 17 biotypes, most DRCNS isolates belonged to one of three major biotypes limiting the usefulness of this method. Ten of the 64 (16%) DRCNS types identified comprised 50 of the 112 (45%) isolates. These were isolated from staff and from patients on day 14, suggesting a nosocomial origin.

INTRODUCTION

Coagulase-negative staphylococci (CNS) belonging to the normal skin flora of humans today cause a wide range of hospital-acquired infections with increasing morbidity and mortality [1]. *Staphylococcus epidermidis* is the predominant species, accounting for 80–96% of CNS infections, but *S. haemolyticus*, *S. warneri*, *S. cohnii*, *S. capitis*, *S. simulans*, *S. xylosus*, *or S. hominis* may also be involved [2–6].

Currently, nosocomial isolates of CNS show a high rate of multiple drug resistance, mainly due to acquisition of extrachromosomal DNA [7 9]. Of particular concern is the rapid emergence of chromosomally mediated resistance to methicillin and other beta-lactam agents among CNS. The local drug resistance

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patterns in CNS probably mirror the use of antibiotics in primary health care and especially in the hospital [10, 11]. Hospitalized individuals, in particular patients receiving antibiotics, are reportedly prone to pick up drug-resistant CNS (DRCNS) from the hospital microflora [12–16].

Their growing clinical significance and drug resistance, which narrows the choice of therapy and prophylaxis of CNS infections, has made it increasingly important to understand better the hospital epidemiology of DRCNS. Unfortunately, traditional methods of CNS typing, such as antibiogram and phage typing, have proved to yield moderate discrimination and low typability, respectively, and therefore, are of modest practical value [17–19]. Although plasmid DNA profiles are considered to be useful for discriminating between strains of CNS, combinations of different typing methods have been suggested for improved epidemiological information [18, 20–23].

The objectives of the present study were to evaluate four CNS typing methods and to characterize epidemiologically skin DRCNS in both orthopaedic patients and ward staff. The finger-printing of DRCNS isolates was done by (*a*) species determination, (*b*) a computerized 96-reaction biotyping method originally developed for *Escherichia coli* [24], (*c*) plasmid profile analysis without removal of chromosomal DNA and (*d*) antibiogram typing.

METHODS

Study populations

Sixteen patients admitted to one orthopaedic ward at the County Hospital in Västerås, Sweden, March–April 1987, and discharged after a minimum 14 days stay, were studied. None of them suffered from any skin condition, had been hospitalized, or had received antibiotic therapy during the month prior to admission. Seven patients (six females) received surgical therapy of fractured hip ($n = 5$) or of the spine ($n = 2$), but did not receive any antibiotics during the study period (*Group A*; mean age 68·9 years, range 56–90). Nine patients (four females) underwent hip or knee arthroplasty and received dicloxacillin prophylaxis (Diclocil® Bristol-Myers, 1 g IV t.i.d. on the day of operation followed by 500 mg t.i.d for 2 days post-operatively) (*Group B*; mean age 62·4 years, range 50–78). No additional antibiotics were given during the study period. About one third of the members of the orthopaedic ward staff ($n = 8$) were included for comparison (*Group C*; mean age 38·3 years, range 25–49).

Sampling of drug-resistant coagulase-negative staphylococci (DRCNS)

The anterior nares, perineum, and toe web of the patients were sampled preoperatively within 24 h and post-operatively on day 14 after admission. Each staff member was similarly sampled once during the 8-week study period. Sterile cotton-tipped swabs (Kemi-Intressen, Sundbyberg, Sweden) were premoistened with sampling solution (sterile 0·075 M phosphate buffer containing 0·1 % Tween 80, [25]). A swab was introduced into one nostril, rotated 10 times in each direction, rinsed in 3 ml sampling solution, and again used for sampling of the other nostril. A 4 cm^2 area in the perineum and the fourth left toe interspace were similarly sampled with circular swab motions (10 times in each direction, [10]). All specimens were cultured within 1 h of sampling.

Isolation of DRCNS

Portions (100 μl) of undiluted sample solution and 10- and 100-fold dilutions thereof in 0·05% Tween 80 [10] were plated on six selective PDM–ASM agar (AB Biodisk, Solna, Sweden) plates, each supplemented with one of the following antibiotics: gentamicin (5 mg/l), erythromycin (5 mg/l), clindamycin (5 mg/l), vancomycin (10 mg/l), fusidic acid (10 mg/l), or chlorhexidine (500 mg/l). Also blood agar (Blood agar base 2, LabM, Solna, Sweden) plates supplemented with 4·5% NaCl and methicillin (10 mg/l) were similarly inoculated.

Plates were incubated for 48 h at 37 °C aerobically (30 °C for methicillin plates). From each plate 1–3 colonies of different colonial morphology, each resembling staphylococci, were collected and identified to the genus level by standard laboratory techniques. CNS and *S. aureus* were distinguished by latex agglutination of cell-bound coagulase (Staphy-Slide, Bio-Mérieux), 302 of 488 colonies collected were CNS.

Antimicrobial susceptibility testing

Each of the DRCNS colonies isolated from the drug-containing plates was further tested for susceptibility to various antibiotics by agar disk diffusion using PDM–ASM plates and paper disks (AB Biodisk) containing vancomycin 5 μg, ciprofloxacin 5 μg, rifampicin 30 μg, fusidic acid 50 μg, amikacin 30 μg, gentamicin 30 μg, clindamycin 15 μg, erythromycin 15 μg, trimethoprim/sulphamethoxazole 1·2 + 23·8 μg, or chloramphenicol 30 μg [26]. DRCNS isolates from the same body site were classified as different if the diameters of the antibiotic inhibition zones differed by > 3 mm for at least one agent. This limit was chosen because repeated testing of isolates yielded zone diameter differences of up to 3 mm. Resistance to methicillin was tested by plating on methicillin plates (see above).

Production of beta-lactamase was tested using chromogenic cephalosporin disks (Nitrocefin®, AB Biodisk).

After discarding isolates from each site classified as duplicates, 112 DRCNS isolates considered as potentially distinct were further studied.

Epidemiological marker systems

Species identification. The 112 DRCNS isolates were identified according to Kloos and Schleifer [27].

Biochemical typing. DRCNS were exposed to 96 different biochemical reagents (62 carbohydrates, 22 organic acids, and 12 nitrogen compounds) in microplates including all 13 carbohydrates used by Kloos and Schleifer and the pH indicator bromothymol blue as previously described for *E. coli* [24]. Briefly, the kinetics of each reaction was measured optically at intervals during 48 h using a Titertek Multiskan reader (model MCC/304, Flow laboratories, Inc.) connected to a microcomputer. After the last reading, the sum of all readings for each reaction was calculated, yielding a set of 96 numbers for each isolate (the biochemical fingerprint). These numbers were used for computerized calculations of the correlation coefficients between fingerprints of all strains compared pairwise. A correlation coefficient of $> 0{\cdot}98$ was obtained for duplicate tests of the same isolate and was taken to define biotype identity between isolates.

Plasmid typing. A method for preparation of genomic DNA from *S. aureus* [28]

Table 1. *Antibiogram coding of CNS isolates*

	Code					
	Digit 1			Digit 2		
Antibiotic	FU	GM	CL	EM	TS	CH
Number if sensitive or intermediate	1	2	4	1	2	4
Possible triplet number	0 – 7			0 – 7		
Possible code number	00 – 77					

Abbreviations: FU, fusidic acid; GM, gentamicin, CL, clindamycin; EM, erythromycin; TS, trimethoprim/sulphamethoxazole; CH, chloramphenicol. For methicillin-resistant CNS the prefix MR was added to the two-digit code.

was modified for plasmid DNA preparation. 0·8 ml of an overnight culture in Cy medium [29] was centrifuged for 2 min. The pellet was suspended in 50 μl of 20% sucrose (detergent-free) in Tris-HCl buffer (0·05 M, pH 7·2) and incubated with lysostaphin (100 μg/ml) at 37 °C for 30 min before transfer to ice and addition of 50 μl of lysis solution (0·1% Triton X-100, 0·066 M EDTA and 0·05 M Tris-HCl, pH 8·0). The suspension was gently mixed by inversion of the tube and incubated for 30 min at 20 °C or until lysis occurred, then centrifuged at 20000 rev./min for 15 min. The supernatant was removed and SDS was added to a final concentration of 0·5%. The plasmid DNA preparations of the isolates thus obtained were compared by agarose gel electrophoresis. Preparations with similar plasmid profiles were re-electrophoresed side by side several times in order to establish identity or non-identity. None of the isolates had only 1–2 plasmids of similar size requiring restriction enzyme digestion of plasmid DNA for assessment of identity.

Antibiogram typing. Inhibition zone diameters were translated into resistant, intermediate, or sensitive [30]. For the six most discriminatory drugs (fusidic acid, gentamicin, clindamycin, erythromycin, trimethoprim/sulphamethoxazole, and chloramphenicol) the sensitive–intermediate category was given a numerical designation of 1, 2, or 4. By adding the numbers in each group of three antibiotics, a two-digit code for each isolate was obtained, each representing a unique susceptibility pattern (Table 1). The codes of methicillin-resistant CNS were given the prefix MR. For evaluation of reproducibility of antibiogram codes, 20 random isolates were tested on two occasions.

Statistical analysis

The χ^2-test or Fisher's exact test was used for comparison of proportions.

RESULTS

Drug resistance of CNS flora

The 112 DRCNS isolates considered to be potentially distinct were collected from 52 body sites in 22 of the 24 subjects. All isolates produced beta-lactamase. Throughout the study, none of the CNS sampled was resistant to vancomycin, rifampicin, amikacin, or chlorhexidine; whereas the prevalence of CNS resistant to the other seven agents studied (trimethoprim/sulphamethoxazole, gentamicin,

Table 2. *Drug-resistant CNS (DRCNS) among orthopaedic patients and members of ward staff*

	Isolation of DRCNS (no. of subjects/body sites*)				
	Group A		Group B		Group C
	Day 1	Day 14	Day 1	Day 14	
CNS resistant to†	$n = 5/15$	$n = 7/21$	$n = 9/27$	$n = 9/27$	$n = 8/24$
Any agent	1/1	6/14‡	3/6	9/19§	7/18
6 agents	0/0	3/3	0/0	1/1	0/0
5 agents	0/0	4/8§	0/0	4/4	2/2
Trimethoprim/sulphamethoxazole	0/0	5/12‡	1/2	9/19‡	5/8
Gentamicin	0/0	6/12‡	0/0	8/14‡	5/7
Clindamycin	1/1	5/11§	1/1	6/7	3/5
Erythromycin	1/1	5/12§	3/5	7/12	7/16
Chloramphenicol	1/1	6/12§	0/0	8/12‡	6/6
Fusidic acid	0/0	1/1	0/0	1/1	2/3
Methicillin	1/1	6/8	1/1	7/14‡	4/5

* Group A, no antibiotics; B, dicloxacillin prophylaxis; C, ward staff. *n*, Number of subjects/body sites studied.

† No CNS resistant to vancomycin, ciprofloxacin, rifampicin, amikacin or chlorhexidine were found.

‡ Proportion of body sites with DRCNS as compared to day 1. $P < 0{\cdot}001$.

§ Proportion of body sites with DRCNS as compared to day 1. $P < 0{\cdot}05$.

clindamycin, erythromycin, chloramphenicol, fusidic acid, and methicillin) had increased between day 1 and day 14 in both patient groups (Table 2). On admission only 1 of 5 group A patients and 3 of 9 group B patients carried DRCNS as compared to 7 of 8 among staff; whereas, on day 14, 6 of 7 group A and all of 9 group B patients were colonized with such strains. Similarly, 1 of 15 body sites sampled on admission in group A patients had DRCNS as compared to 14 of 21 at day 14 ($P < 0{\cdot}01$) and 18 of 24 among the staff (Table 2). Among group B patients 6 and 19 of bodysites ($P < 0{\cdot}01$) had DRCNS on admission day and day 14, respectively ($P < 0{\cdot}01$).

On day 14 CNS resistant to 5–6 agents were more prevalent in group A patients than among group B patients or among ward staff (11 of 21 *v.* 5 of 27 *v.* 2 of 24 body sites colonized, respectively, $P < 0{\cdot}05$, Table 2). In contrast, a greater increase in the colonization rate with methicillin-resistant CNS between days 1 and 14 had occurred in group B (1 of 27 *v.* 14 of 27 body sites colonized, $P < 0{\cdot}001$) than in group A (1 of 15 *v.* 8 of 21, $P > 0{\cdot}05$). Thus, although hospitalization was generally associated with colonization with multiple DRCNS, including methicillin-resistant CNS, dicloxacillin prophylaxis shifted the patterns towards resistance against methicillin plus one or only a few other drugs, notably trimethoprim/sulphamethoxazole and gentamicin (Table 2).

DRCNS types

The 112 DRCNS isolates were subject to four different typing methods.

Species. Most DRCNS isolates were *S. epidermidis* (69%) and 21% were *S.*

Table 3. *Types identified among 112 drug-resistant CNS isolates**

Species	Biochemical type	Plasmid type	Antibiogram type	No of isolates
S. hominis	1	NT	76, MR31, MR35, MR55, MR75, MR76	16
		S	10, 75, MR31, MR75	5
	S	S	76, MR36	3
S. cohnii	2	S	76, MR67, MR71, MR74	5
	S	NT	76	1
	S	S	MR36	1
S. warneri	S	S	76	1
	S	NT	76	1
	3	1	MR76	2
S. epidermidis	4	2	10, 14, 51, 67, 71, 73, 76, MR10, MR30, MR71, MR75	42
		3	14, 34	4
		4	75, MR10	3
		S	10, 14, 34, 51, 53, 54, 67, 71, 73, 74, 75, 76, MR76	17
	5	S	76	1
		NT	76	1
	6	5	76, MR76	5
	S	S	76	4
Total no.† 4	17	42	23	112

* S, Sporadic type represented by only one isolate. NT, Plasmid-less and thus not typable. MR, Methicillin-resistant.

† Comprising 64 distinct types when combining all four typing methods.

hominis, 6% *S. cohnii*, and 4% *S. warneri* (Table 3). Of the 37 methicillin-resistant isolates studied only 13 were *S. epidermidis* (17% of the isolates of this species). Methicillin resistance was more common among *S. hominis* (75%, $P < 0{\cdot}001$) and *S. cohnii* (57%, $P < 0{\cdot}05$). Two of four (50%) *S. warneri* isolates were methicillin-resistant.

Biochemical types. With the method used the DRCNS isolates were rather homogenous biochemically, yielding only 17 distinct types (Table 3). Six of these (types 1–6) each comprised 2–66 isolates and represented 90% of all DRCNS isolates, whereas 11 biochemical types occurred as sporadic isolates. The most common type (type 4, *S. epidermidis*) comprised as many as 66 (59%) of all isolates. Seven distinct biochemical types were identified among the 77 *S. epidermidis* isolates, and 2–4 types for each of the other CNS species.

Plasmid types. Plasmids were demonstrated in 93 isolates (83%). In contrast, only 41% of methicillin-resistant isolates could be typed by plasmid profile analysis, mainly because *S. hominis* usually was methicillin-resistant, but plasmid DNA was poorly or not recovered. Of the 42 plasmid types identified, five types (Nos. 1–5) comprised 56 (50%) of the isolates, whereas each of the remaining 37 types was unique to a single isolate (Table 3). The predominant plasmid pattern (type 2) was found only in *S. epidermidis* of biochemical type 4, comprising 64% of such isolates. Plasmid type 2 was associated with 11 distinct antibiogram types

(see below and Table 3), indicating that loss or acquisition of drug resistance genes was often not revealed by plasmid profile analysis.

Antibiogram types. The reproducibility of inhibition zone diameters was only modest (92%, with variability of > 3 mm occurring mainly for gentamicin, data not shown). However, this rarely affected the antibiogram codes (reproducibility 97·5%). Using six antibiotics selected for optimal discrimination (Table 1), a total of 23 distinct antibiogram types were found (Table 3). The four most prevalent types (codes 10, 14, 71 and 76) among the 75 methicillin-sensitive DRCNS comprised 69% of these isolates. Among the 11 types of methicillin-resistant isolates, code MR55 (resistance to methicillin, gentamicin and trimethoprim/sulphamethoxazole) was the most common (24% of such isolates).

In both patient groups twice as many antibiogram types were found in the perineal flora than in the nose and toe web floras on day 14; mean of both groups 2·5 (perineum) *v.* 0·8 (nose) and 1·2 (toe web) codes per individual, respectively ($P < 0{\cdot}05$). In contrast, the most diverse DRCNS flora among staff members was found in the toe web samples with a mean of 2·8 antibiogram codes per individual.

Combination of typing methods

Application of species determination, biochemical, plasmid, and antibiogram typing singly or in combinations, yielded typabilities of 83–100% and 4–64 types among the 112 DRCNS isolates studied (Table 4). Although the combination of all typing methods as well as only the three latter together yielded the maximum number of types, plasmid, and antibiogram typing resulted in nearly as many (61, Table 4). Plasmid typing alone yielded 42 types and 46 types when combined with species determination and biochemical typing. Species determination plus antibiogram typing and antibiogram typing alone distinguished only 33 and 23 types, respectively. Thus, both plasmid and antibiogram typing were essential for optimal discrimination, whereas species determination and biochemical typing were of little additional value.

The most common antibiogram type (76, resistance to erythromycin, 30/112 isolates, 27%) was also the most dispersed and occurred in 19 (30%) of all 64 types identified.

DRCNS epidemiology

On admission, the patients carried only 9 of all 64 (14%) DRCNS types identified (0·6 per individual, Table 5). This carrier rate had increased to 4·4 in group A (31 isolates) and 4·7 in group B (42 isolates) on day 14. *S. epidermidis* of biochemical type 4 and plasmid type 2 was found only twice on admission, but by day 14 had colonized 6 of 7 group A and 6 of 9 group B patients, accounting for 41% of patients DRCNS isolates.

Orthopaedic ward staff members (group C) carried 3·8 DRCNS types per individual (30 isolates; Table 5). Again, S. epidermidis, biochemical type 4, plasmid type 2 was found in 6 of 8 individuals and accounted for 9 of 33 (27%) of the isolates.

Ten of the 64 (17%) types of DRCNS identified could be classified as nosocomial since they were found both among staff and among patients on day 14 but never among patients on day 1 (Table 5). These presumed hospital DRCNS types had,

Table 4. *Outcome of typing methods applied singly, or in combination, to 112 drug-resistant CNS from orthopaedic patients and ward staff members*

	Outcome of typing									
	One method				Combinations of methods					
Typing method										
Species	+				+	+				+
Biotype		+				+	+		+	+
Plasmid profile				+			+	+	+	+
Antibiogram			+		+	+		+	+	+
Typability (%)	100	100	100	83	100	100	100	100	100	100
Discrimination, (no of types)	4	17	23	42	33	46	46	61	64	64

Table 5. *Distribution of drug-resistant CNS types among orthopaedic patients and ward staff members*

	No. of subjects/types/isolates*				
	Day 1	Day 14		During study	
	Group A+B $n = 14$	Group A $n = 7$	Group B $n = 9$	Group C $n = 8$	Whole study $n = 24$
Possible nosocomial types†	0/0/0	5/7/14	6/7/20	4/10/16	15/10/50
All types	4/9/9	6/22/31	9/23/42	7/24/30	22/64/112

* Types refers to combined results of species determination, biochemical, plasmid, and antibiogram typing (see Table 4 and text). Group A, no prophylaxis; B, dicloxacillin prophylaxis; C, ward staff. *n*, Number of subjects.

† Isolated from staff members and from patients only in day 14.

by day 14, colonized 5 of 7 group A patients and 6 of 9 group B patients and comprised 47% of the patient isolates and 53% of the DRCNS isolates from staff (Table 5). Other fellow patients or staff members were not evaluated as potential nosocomial sources of DRCNS strains.

DISCUSSION

Drug resistance of CNS flora

This study further elucidates the intricate situation regarding drug resistance and other ecological aspects of the skin CNS flora of hospitalized patients and ward staff. Our patients acquired CNS resistant to trimethoprim/sulphamethoxazole, gentamicin, clindamycin, erythromycin, chloramphenicol, and fusidic acid, and at rates similar to or even surpassing those among staff members. In agreement with others [12–16], we found that patients who received dicloxacillin prophylaxis were more heavily colonized with methicillin-resistant CNS by day 14 of hospitalization than were patients who had not received prophylaxis and ward staff members.

Emergence of bacterial resistance to chlorhexidine, especially among enteric bacteria, has been reported [31]. This was not found among CNS in the present

study, despite long-term routine use of preoperative whole body disinfection using soap containing chlorhexidine. No CNS resistant to vancomycin were found. This was true also for ciprofloxacin, amikacin, and rifampicin, although resistance to the two latter agents in CNS has been reported [32]. Rapid emergence of ciprofloxacin resistance in skin CNS may follow therapy with this agent [33]. The fact that ciprofloxacin had not been introduced into the hospital at the time of this study, probably explains the apparent absence of resistance to this agent.

Typing of DRCNS

Typability is a main criterion for the usefulness of a typing method. Low typability (< 50%) is a continuous and apparently mounting problem with phage typing of CNS [17, 19, 34]. Also, plasmid profile analysis reportedly has variable typability due to the occasional lack of plasmids [19]. In the present investigation, typability was 83% for plasmid typing, but 100% for species determination, biochemical, and antibiogram typing. High typability has been reported also for total DNA and immunoblot fingerprinting [34], but these methods are as yet not readily available.

Reproducibility is another criterion for typing methods. Despite the modest reproducibility of individual zones of inhibition, the antibiogram codes based on breakpoints were highly reproducible (97·5%). These results with those reported by others [18, 23]. Reproducibility was very high also for biochemical typing, but was not evaluated for species determination and plasmid typing.

Discrimination is a third criterion for typing methods. Our biochemical typing method yielded better discrimination of CNS than species determination. This was especially true for *S. epidermidis* isolates among which we were able to distinguish seven distinct biochemical types. Some of these included large numbers of isolates of varying plasmid patterns and antibiogram codes. Biochemical typing thus provided further discrimination of only four plasmidless isolates of identical antibiogram type. Although DRCNS were biochemically rather homogenous and thus contained relatively few biochemical types using our method, this typing method may deserve further development and evaluation.

We identified 42 plasmid types and 23 antibiogram types among the 112 DRCNS isolates studied. This result markedly differed from that of Hartstein and co-workers [23] who suggested that antibiogram alone is equally discriminating as plasmid profile analysis among repeated blood isolates of CNS.

As expected, the maximum number of types (64 types) was obtained when all four typing methods were used, but also when species determination was omitted. However, antibiogram and plasmid typing yielded nearly as many types (61 types), and probably represents the most discriminating routine typing strategy for CNS currently available, as also found by other workers [35].

Nosocomial epidemiology of DRCNS

When applying all four epidemiological marker systems to the DRCNS flora of orthopaedic surgical patients and ward staff members, a remarkably diverse and complex picture emerged. Several distinct DRCNS types per subject were identified of which 10 types were of apparent nosocomial origin. These latter types may have been picked up from the staff since they were recovered from patients

only after 14 days hospitalization and from the staff. However, it cannot be excluded that they were present on admission on the patients skin at body sites not sampled and thus escaped detection. Furthermore, because of the limited size of the study population (comprising only part of all patients and one third of staff members), the precise origin of nearly half of the DRCNS types, which colonized the hospitalized patients, remains unknown. However, our data suggest that new colonization by different nosocomial DRCNS types rather than acquisition of plasmids and drug resistance genes by resident strains was the major mechanism of the rapid emergence of DRCNS during hospitalization.

In summary, the present study shows that of four typing methods studied, the combined use of plasmid and antibiogram typing yielded near maximal discriminatory ability among DRCNS isolates. Using this tool a highly diverse DRCNS skin flora with a rapid and complex spread in an orthopaedic ward was demonstrated.

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