Methicillin-resistant *Staphylococcus aureus* (MRSA): isolation from nasal and throat swabs transported in liquid or semisolid media; identification by PCR compared with culture

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Abstract

Objectives: A multi-site study evaluated the clinical performance of the Roche LightCycler PCR assay in the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in comparison to routine chromogenic agar culture method (BD BBL CHROMagar II). Two other variables were also investigated: to establish if screening the throat increases the detection rate of MRSA colonisation; and, to determine MRSA isolation rates from swabs transported in semi-solid media (Copan Venturi Transystem) and liquid-based media (Copan ESwab).

Methods: 810 swab specimens were collected from the anterior nares and throat to determine the site specific isolation of MRSA. Swabs were transported in liquid (Copan Elution swab) and semi-solid (Copan Venturi Transystem without charcoal) transport media to assess the effect of collection swab type on the viability of MRSA. Swabs specimens were screened for MRSA using chromogenic (direct and broth enriched) and PCR techniques.

Results: MRSA incidence among volunteers was 2.6% (culture) and 4.8% (PCR). The 2.2% higher yield obtained by the PCR method was statistically significant ($P = 0.04$). This increase in sensitivity incurs an additional cost (per test, the PCR assay was 2.5 times more expensive). However, the PCR method had a much faster result turnaround time (2-3 hours) compared to culture (48 hours) while both methods had comparable sample hands on time (1.65 min and 1.20 min, respectively).

Conclusions: The throat was found to be an important habitat of MRSA. In this study, if only the nares were tested, 38.5% (PCR) to 42.8% (culture) of the total MRSA carriers would have been missed. Thus, any screening program for MRSA should include swab specimens from the throat. Both swab types performed almost equally in maintaining the viability of MRSA during the study. While the elution swab was approximately double the price of the Venturi counterpart, the liquid phase allows the pooling of samples and multiple testing to be performed from one swab specimen.

Key words: methicillin-resistant *Staphylococcus aureus,* MRSA, polymerase chain reaction, chromogenic culture, mass screening, infection control, microbial viability.

Introduction

Staphylococcus aureus is an opportunistic pathogen carried as a commensal organism on the skin and in nares of approximately 30% of the normal population (1). *S. aureus* is responsible for a broad range of clinical infections, most notable of which are cases of bacteraemia and endocarditis (2).

Methicillin-resistant *S. aureus* (MRSA) was first described in 1961, with the first hospital outbreak reported in 1963 (3). Healthcare associated infections caused by MRSA have become an important issue for healthcare facilities worldwide due to high rates of infection, mortality, and high costs of treatment (4). During 2005, it was estimated that invasive MRSA infections in the United States resulted in almost 19,000 deaths, a figure higher than that for HIV/AIDS (5).

Laboratory-based screening for MRSA colonisation in patients and healthcare workers remains a cornerstone of infection control measures to limit the spread of MRSA (6). Factors that determine the success of a screening program include: the efficiency of a collection systems to maintain viability and recovery of organisms (7); the sensitivity and speed of laboratory testing (8,9); the speed of result determination (8-10); and sampling site or sites (11,12). To rapidly detect MRSA colonisation in patients, laboratories must choose between molecular methods and selective agar-based methods (13). In high-risk patients, the rapid detection of MRSA can be of double value: not only are they in the interests of patients infected with MRSA (in order to start adequate treatment as early as possible), but they also help to protect other patients from potentially spreading the pathogen.

The use of chromogenic media in recent years has become an important method for the rapid identification of microorganisms in clinical samples (14). In comparison to conventional culture media, chromogenic media allows direct colony colour-based identification of the organism from the primary culture. This in turn, reduces the need to subculture for further biochemical testing and hence the time until a result is obtained (15). The advantages of using BBL CHROMagar II media include: short hands on time, minimal personnel time required for reading cultures, and the low cost of the test in comparison to PCR methods. The disadvantage with using this media is the long turnaround times. Several chromogenic and differential MRSA media have been shown to yield results within 18 to 48 hours (16-18). In contrast, PCR methods can yield results within two to three hours (8,11,13).

In 2008, Roche Molecular Systems (Switzerland) introduced the LightCycler (LC) MRSA advanced test, an *in vitro* diagnostic PCR method for the rapid detection of MRSA colonisation to aid in the prevention and management of MRSA infections in healthcare settings. The test targets the integration site of the SCC*mec* cassette into the *S. aureus* chromosome with melting point analysis of the PCR product (8,11). This PCR assay is performed on the LC 2.0 (Roche Applied Science) instrument, an extremely fast thermocycler with on-line fluorescence detection. The shortfall with PCR techniques however, is that the emergence of new MRSA strains with diverse genetic background also means that the capability of PCR testing will need to be constantly updated in order to detect SCC*mec* cassettes with novel sequences (19).

For MRSA detection, swabs are the most commonly used sampling device. These systems must maintain organism viability during transit while allowing maximal organism recovery during laboratory testing. Given the increasing frequency of transport delays due to cost containment measures, consolidations, and services being shifted to centralised or reference laboratories, robust transport systems are becoming increasingly important. The vast majority of swab transport systems used in New Zealand and the rest of the world contain a semi-solid medium which is reported to give better survival of organisms, particularly of anaerobes (20).

A new type of swab system has recently been introduced in a growing number of laboratories (21). The Copan Elution Swab (ESwab) consists of a sterile package containing two components: a pre-labelled polypropylene screw-cap tube with conical shaped bottom filled with 1mL of liquid Amies transport medium and a specimen collection swab which has a tip flocked with soft nylon fibre (22). This swab is prepared by spray-on flocked fibre technology which provides stronger capillary action and strong hydraulic uptake of liquids, which should result in better specimen collection (23). Organisms present in the specimen remain close to the surface and when placed in transport medium, elute completely and immediately (24). An extra advantage of the liquid medium over conventional swabs is that every inoculated plate receives a similar inoculum (20).

To date, current New Zealand MRSA screening programmes do not include throat swabs as specimen of choice. Such samples are thought to add discomfort to the patient during collection with little anticipated gain. This belief is based on the idea that throat carriers of *S. aureus* are likely to carry *S. aureus* in the nares as well. However, several studies have shown individuals may have colonisation exclusively in the throat that would be missed on screening limited to the anterior nares only (25-29). Additionally, the existence of different clones of *S.aureus* in the nares and in the throat has been confirmed (25). This supports the notion that staphylococcal flora in the nose and the throat are independently formed and that attention should also be directed to the carriers of *S. aureus* in the throat for the control of nosocomial infection (30). Unrecognised carriers may spread MRSA and render infection control programmes futile; therefore one must question the practice of screening of the anterior nares alone and evaluate the additional benefit of screening both the nares and the throat.

The purposes of this multi-site study were to: determine if screening the throat increases the detection rate of MRSA colonisation; to determine the performance of the Roche LightCycler MRSA advanced test in the detection of MRSA in comparison to routine culture on selective chromogenic agar (BD BBL CHROMagar II); and, to determine MRSA isolation rates from swabs transported in semi-solid media (Copan Venturi Transystem) and liquid-based media (Copan ESwab). The *spa* type and MRSA strain (if the *spa* type indicated that the MRSA isolate belonged to a named strain) were reported. *spa* typing could only be performed on cultured isolates.

Materials and methods
Swab specimens were collected from volunteers between June and December 2011. Subject samples were collected at nine sites across New Zealand. Specimens were tested at one site in Palmerston North, New Zealand. The volunteers consisted of: patients from hospital non-intensive units; medical laboratory staff; and hospital medical staff including nurses, physicians, infection control and administration staff. All volunteers were ≥16 years of age and provided written informed consent to participate in the study. Exclusion criteria included (i) antibiotic therapy, either topical or systemic that is active against MRSA up to three months prior to sample collection (ii) previous enrolment in the study (iii) contraindications to nasal and throat sampling according to the institution's policy.

This study was conducted in accordance with the New Zealand Health and Disability Ethics Committee multiple-site guidelines (Ethics reference number: CEN/11/EXP/030).

Specimen collection
Three swabs were collected from each subject, two nasal and one throat swabs. Nasal specimens were collected using two swab types: (i) Venturi Transystem rayon-tipped swab with Amies gel transport media (Copan, Italy), and (ii) Elution Swab (ESwab), a nylon-tipped flocked swab with 1mL of liquid Amies transport media (Copan, Italy). For throat specimens, only the ESwab was used. For collection, the anterior nares were sampled by insertion of the swab tip approximately one inch into the nostril and rotated against the mucosa five times. The same procedure was then repeated for the second nostril using the same swab. The throat was sampled by rotating the swab tip on both tonsils. Swabs of the anterior nares were collected first and then, using a different swab, the throat was sampled. The swabs were transported to the laboratory in their respective transport containers.

Experimental design
Nasal and throat ESwab swab specimens were tested by the Roche LC MRSA advanced PCR test, and the results were compared to those obtained by direct culture on BD BBL CHROMagar II MRSA medium (Fort Richard, Auckland) and broth-enriched culture containing 6.5% NaCl (salt/enrichment broth, Fort Richard, Auckland) followed by culture on BD BBL CHROMagar II MRSA media (referred to as CHROMagar from here on in). The nasal Venturi swabs were not tested on the LC. Swabs were cultured on the day of collection and batched in a run of 30 specimens for the LC PCR test. Swab samples were frozen (-20 ±2°C) after direct culture if a batch of 30 specimens was not available.

Electronic timers were used to monitor the start and finish times for sample preparation, working master-mix preparation, and the PCR preparation for the LC PCR test. Similarly, for CHROMagar culture, electronic timers were used to document the times for media inoculation, streaking, broth sub-culture of the samples and the final reading for directly plated and enrichment cultures. Times to result for all samples included in the study were also documented and are presented as mean times.

Culture methods
All three swab heads were cultured by rolling the swab head directly onto CHROMagar MRSA medium. The plates were incubated for a total of 48 ±4 hours (35 ±2 °C), with examinations at 24 ±4 hours and 48 ±4 hours of incubation. The ESwab heads were then processed using the LC PCR test.

Subsequently, the Venturi Transystem swab head and a 0.5 mL aliquot of the Liquid Amies transport media from each ESwab were individually inoculated into a vial containing 1mL 6.5% salt broth. Following incubation for 18-24 hours (35 ±2 °C), the broth was sub-cultured onto another CHROMagar MRSA plate, and the plate was incubated for 24 ±4 hours as described above.

Molecular methods
The Roche LightCycler MRSA advanced test was performed according to the manufacturer's instructions (Roche Diagnostics, Switzerland). This assay targets the integration site of the SCCmec cassette into the *S. aureus* chromosome. Essentially, the LC PCR assay is performed on the LightCycler 2.0 instrument and has been validated with three transport media (Liquid Stuart, and Amies gel with or without charcoal).

MRSA isolation and confirmation
After incubation, growth of mauve colonies were considered positive (indicating MRSA) and no growth or colonies with other colours were considered negative.

The positive isolates were inoculated onto nutrient agar slopes (Fort Richard, Auckland), incubated at 35 ±2°C for 24 ±4 hours, and stored at room temperature for additional testing. To confirm MRSA, suspected

isolates underwent a tube coagulase test (Remel) and cefoxitin resistance screening using the Clinical Laboratory Standards Institute (CLSI) disk diffusion method (31). Colonies were suspended in tryptic soy broth (TSB, Fort Richard, Auckland) to a turbidity of 0.5 McFarland standard, plated on Mueller-Hinton agar (Fort Richard, Auckland), and a 30 µg cefoxitin disc (Thermo Fisher Scientific, Auckland) was placed within the inoculum. Inhibition zone diameters were measured and recorded after a 24 ±4 hours incubation at 35-37°C (susceptible, ≥22mm; and resistant, ≤21mm).

PCR positive swab specimens that were culture negative were confirmed using the Qiagen DNAeasy Blood & Tissue Kit (Qiagen Sciences, MD). This assay tests for the presence of *mec*A using a method proposed by Huletsky et al. (32). A set of PCR primers specific to the different SCC*mec* right extremity sequences (mecii574, meciii519, meciv511, mecv492, and mecvii512) as well as a primer specific to *S. aureus orfX* (Xsau325) were used in combination with three molecular beacon probes (XsauB5-FAM, XsauB8-FAM, and XsauB9-FAM) targeting *orfX* sequences (32). The PCR reaction mix for this assay contained the following: 1x PCR buffer (minus Mg), 0.2 µM dNTP, 1.5 mM MgCl$_2$, 0.2 µM of each primer 1.0 unit of Platinum Taq DNA polymerase, template DNA and sterile MQ water (to make up to 50 µl). The PCR products were run on a 1.5% agarose gel (containing ethidium bromide) in 0.5X TBE buffer at 100 Volts for 75 minutes. The bands were visualised using the Multi Doc-It Digital Imaging System (UVP, CA).

Antimicrobial susceptibility testing
To characterise MRSA strains, coagulase positive isolates determined to be resistant to cefoxitin (i.e, MRSA) underwent antibiotic susceptibility testing using the CLSI method described above, for the following antibiotics (Thermo Fisher Scientific, Auckland): penicillin (10 µg), rifampicin (5 µg), ciprofloxacin (5 µg), co-trimoxazole (25 µg), erythromycin (15 µg), clindamycin (10 µg) fusidic acid (10 µg), gentamicin (10 µg), mupirocin (5 µg), and tetracycline (30 µg). Except for fusidic acid and mupirocin, zones of inhibition were interpreted according to CLSI criteria (33). Fusidic acid zones of inhibition were interpreted as: ≥21 mm susceptible; 20 mm intermediate; and ≤19 mm resistant (34). Mupirocin zones of inhibition were determined with a 5 µg disc and interpreted as: ≥14 mm susceptible, and ≤13 mm resistant (35). Additional testing included the determination of oxacillin minimum inhibitory concentration (MIC, 256-0.015 µg/mL) and the detection of inducible clindamycin resistance using the CLSI reference methods (31). *S. aureus* ATCC 29213 was the positive control. The isolates were then inoculated onto nutrient agar slopes, incubated (35°C for 24 h) and sent to the Institute of Environmental Science and Research, Porirua, New Zealand (ESR) for staphylococcal protein A gene (*spa*) typing.

Staphylococcal protein A (*spa*) typing
ESR sequence the *spa* gene using a method adapted from that described by Strommenger et al. (36). *spa* sequences were analysed using Ridom StaphType software version 2.0.3 (Ridom GmbH, Würzburg, Germany).

Statistical analysis
Statistical significance was determined by the McNemar's test. A *P* value of ≤0.05 was considered statistically significant.

Results
During the study period from June to December 2011, 810 specimens were collected from 270 eligible volunteers enrolled at the nine clinical study sites. In total, 91% of volunteers were aged 16-60 years, and 9% were aged >60.

The median age group was 40-49 years. Females were over-represented, comprising 73% of the total volunteers. The majority of specimens were collected from medical laboratory workers (89%), followed by specimens from medical staff (9%), and hospital patients (2%).

Comparison of culture and PCR for the detection of MRSA
From a total of 270 volunteers screened, the CHROMagar culture method (direct and broth enriched) detected seven (2.6%) MRSA positive volunteers and the Roche LC PCR test detected 13 (4.8%) MRSA positive volunteers (Figure 1). The 2.2% higher yield obtained by the PCR method was statistically significant (*P* = 0.04). MRSA isolation via broth enriched culture was considerably higher (2.6%) than direct culture (1.5%). While females were over-represented in all groups except the hospital patient group, no significant difference in isolation rates was observed between males and females (data not shown). MRSA was isolated from one hospital patient. The remaining MRSA positive samples were isolated from laboratory workers.
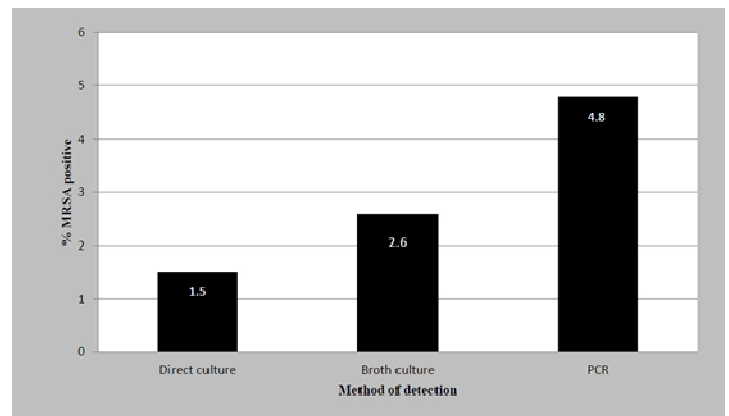
Figure 1. MRSA positivity rate among the 270 volunteers screened for MRSA using: the Roche LC test (PCR); culture on BD CHROMagar (direct culture); and culture in salt broth followed by subculture onto BD CHROMagar (broth culture).

Among the 540 pairs of swabs tested by PCR and culture, 10 were positive by culture and 16 were positive by the LC assay. The 10 specimens positive by culture were from seven volunteers, and the 16 MRSA PCR positive specimens were from 13 volunteers, all of whom had two swab specimens tested with each method (one nose and one throat swab). Table 1 summarises the efficiency of the LC assay in comparison with culture for the detection of MRSA. Of the six specimens from six volunteers that were PCR positive but culture negative, none were culture-positive after subculture from the enrichment broth, but

Table 1. Sensitivity, specificity, positive predictive value, and negative predictive value of the Roche LC MRSA PCR assay in comparison with the BD CHROMagar culture method (direct and salt broth culture combined- composite gold standard) for the detection of MRSA from nose and throat swabs collected from 270 volunteers.

| PCR	Culture (composite gold standard)		Compared with culture			
	MRSA positive	MRSA negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Positive	10	6				
Negative	0	524	100	98.9	62.5	100
PPV = positive predictive value. NPV = negative predictive value.						

Site-specific isolation of MRSA

Of the 270 volunteers studied, the percentage of MRSA carriers considering the nares, the throat and both sites, using both the PCR and culture methods, was 4.8% (13 volunteers). The incidence of MRSA colonisation from each site is displayed in Table 2.

Table 2. Swabs from the anterior nares and throats of 270 volunteers were screened for MRSA by the Roche LC test (PCR) and by culture on BD CHROMagar (direct and salt broth culture combined).

	PCR method			Culture method	
	Anterior nares			**Anterior nares**	
Throat	Positive	Negative	**Throat**	Positive	Negative
Positive	3	5	Positive	3	3
Negative	5	257	Negative	1	263

The Roche LC PCR assay displayed superior performance compared to the culture method in the detection of MRSA from volunteers with single site colonisation, especially the anterior nares. However, the PCR method had equal performance to the culture method in detecting MRSA from volunteers with both nose and throat colonisation of MRSA (Figure 2).
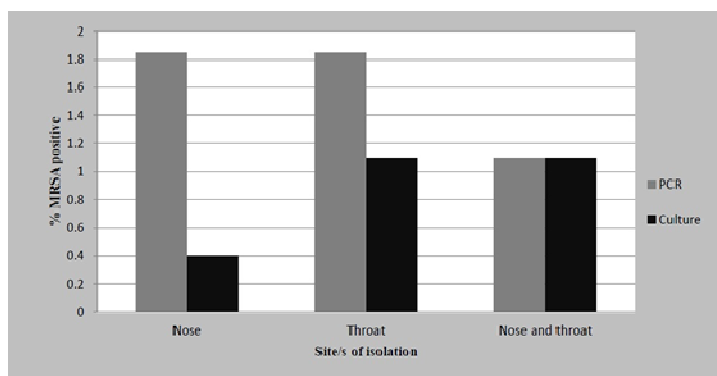
Figure 2. MRSA positivity rate from the anterior nares and/or throats of the 270 volunteers screened for MRSA using the Roche LC test (PCR) and the CHROMAgar culture method (direct and salt broth culture combined).

When the PCR assay was used, throat only MRSA carriers accounted for 38.5% of the total MRSA isolations, equal to that isolated from nasal-only carriers. Dual site colonization accounted for the remaining 23%. However, when culture was employed, 42.8% of the total MRSA positive volunteers were throat only carriers, equal to dual site carriers. The remaining 14.4% were nose only carriers, less than half the figure detected using the PCR assay (Table 3).

Table 3. MRSA isolation from the different sampling sites (nose and/or throat) among the MRSA positive volunteers using the Roche LC test (PCR) and the BD CHROMagar culture method (direct and salt broth culture combined).

Type of carrier	% (#) of total MRSA carriers	
	PCR	**Culture**
Throat only	38.5 (5)	42.8 (3)
Nasal only	38.5 (5)	14.4 (1)
Nasal and throat	23.0 (3)	42.8 (3)

Comparison of swab type on the viability of MRSA

From the seven MRSA culture positive volunteers, one tested positive using the ESwab that was missed using the Venturi swab. This was re-tested and confirmed. In regards to the remaining negative MRSA culture results, the ESwab and the Venturi swab systems were 100% concordant.

Turn-around time performance

The technical hands on time as well as the mean time to result for the Roche LightCycler MRSA advanced test and CHROMagar culture are presented in Table 4. For both methods, the labour involved was documented for 30 specimens and then averaged for a per sample value. The culture labour calculations included: inoculation and streaking of media; enrichment broth inoculation; 24-hour culture interpretation; broth sub-culture; and 48-hour culture interpretation. Time for confirmatory testing was not included. For the LC PCR assay, calculations included: lysate preparation; working master mix preparation; and specimen and control PCR preparations.

Table 4. Mean processing times and time to result of the PCR assay and culture for a batch of 30 samples. 27 batches were tested by the culture method using the 810 swab samples collected from 270 volunteers. 18 batches were tested by the PCR method using the 540 swab samples collected from 270 volunteers.

Time for assay/test	Mean time ± SD
Roche LightCycler MRSA Advanced Test (PCR)	
Lysate preparation	27 ± 3.7 min
Master mix preparation	2.6 ± 0.3 min
PCR preparation	20 ± 3.0 min
Amplification and detection	79.0 ± 2.4 min
Total hands on time per run	49.4 ± 5.2 min
Total time per run (time to result)	2.14 ± 0.1 hours
Average hands on time per specimen	1.65 ± 0.17 min
BD BBL CHROMagar II culture	
Swabbing, streaking, and broth inoculation	36.7 ± 3.4 min
Direct culture- 24-h reading (time to result)	24 ± 4 hours
Salt broth culture- 48-h reading (time to result)	48 ± 4 hours
Average hands on time per specimen	1.20 ± 0.1 min

Regardless of the result, the average personnel hands-on-time was 1.65 min per sample for the LC PCR assay, which was comparable to that of culture value of 1.20 min per sample. However, the average time to get the result for the PCR technique was considerably shorter than that of culture (2.14 and 48 hours respectively).

Antimicrobial resistance and strain variation

MRSA isolates were penicillin (100%), erythromycin (10%), clindamycin (10%), ciprofloxacin (0%), mupirocin (20%), rifampicin (0%), gentamicin (0%), tetracycline (0%), fusidic acid (20%), and co-trimoxazole (0%) resistant. Table 5 summarises the antimicrobial resistance, melting temperatures (T_M), and strain variation between the MRSA isolates.

Table 5. Antimicrobial susceptibility patterns, melting temperatures (Tm, °C), *spa* types, MRSA strains, and SCC*mec* types of the 10 MRSA cultures isolated by the culture method (direct and salt broth culture combined).

Isolate	Site	Fox	Ox MIC*	E	DA	CIP	FD	MUP	PEN	SXT	RD	TET	CN	Tm (°C)	*spa* type	Strain	SCC*mec* type
A1	Nose	R	32	S	S	S	S	S	R	S	S	S	S	58.1	t002	AK3	IV
A2	Throat	R	32	R	R	S	S	S	R	S	S	S	S	58.1	t002	AK3	IV
B	Throat	R	4	S	S	S	S	S	R	S	S	S	S	58.7	t5677	No strain	-
C	Throat	R	4	S	S	S	S	S	R	S	S	S	S	59.6	t019	WSPP	IV
D	Nose	R	4	S	S	S	S	S	R	S	S	S	S	58.9	t5677	No strain	-
E1	Nose	R	64	S	S	S	S	S	R	S	S	S	S	59.6	t019	WSPP	IV
E2	Throat	R	64	S	S	S	S	S	R	S	S	S	S	59.6	t019	WSPP	IV
F	Throat	R	64	S	S	S	S	S	R	S	S	S	S	58.7	t5677	No strain	-
G1	Nose	R	32	S	S	S	R	R	R	S	S	S	S	57.7	t127	WR/ AK1	IV
G2	Throat	R	32	S	S	S	R	R	R	S	S	S	S	57.7	t127	WR/ AK1	IV

*Oxacillin minimum inhibitory concentration (ug/mL)

Fox, cefoxitin; E, erythromycin; DA, clindamycin; CIP, ciprofloxacin; FD, fusidic acid; MUP, mupirocin; PEN, penicillin; SXT, co-trimoxazole; RD, rifampicin; TET, tetracycline, CN, gentamicin.

Three volunteers who were nose and throat MRSA positive carried the same MRSA strain in both sites. The only discrepancy was that in volunteer A, the throat strain was resistant to both erythromycin and clindamycin (induced by erythromycin, as was shown by a positive induction test result, i.e. a "D-shaped" zone was observed between the erythromycin and clindamycin discs), while the nasal counterpart was sensitive to both antibiotics. No pattern was evident to suggest that certain strains inhabit certain sites. However, a link between the MRSA strain and the melting temperature was apparent: the WSPP (Western Samoan Phage Pattern) strain had the highest T_M, followed by the AK3 strain, and lastly, the WK/AK1 strain has the lowest T_M. Additionally, the WSPP strain was the most frequently isolated strain, followed equally by the AK3 and WK/AK1 strains. Three *spa* patterns were not related to any identifiable *spa* types.

In total, six swabs were MRSA PCR positive/culture negative. The Qiagen DNAeasy PCR assay was used to confirm the LC PCR assay. All of the six samples were positive for the *mec*A gene and generated fragment lengths of approximately 278 base pairs (Figure 3 and Table 6). *mec*A positive PCR results can have band sizes of 176 , 223, or 278 bp (32).

Table 6. Qiagen DNAeasy PCR assay gel layout.

Well number	Identification
1	1 kb DNA Ladder (Promega) 5
2	Blank
3-8	Samples 1-6
9	Positive Control
10	Negative Control
11	Blank
12	1 kb DNA Ladder (Promega)
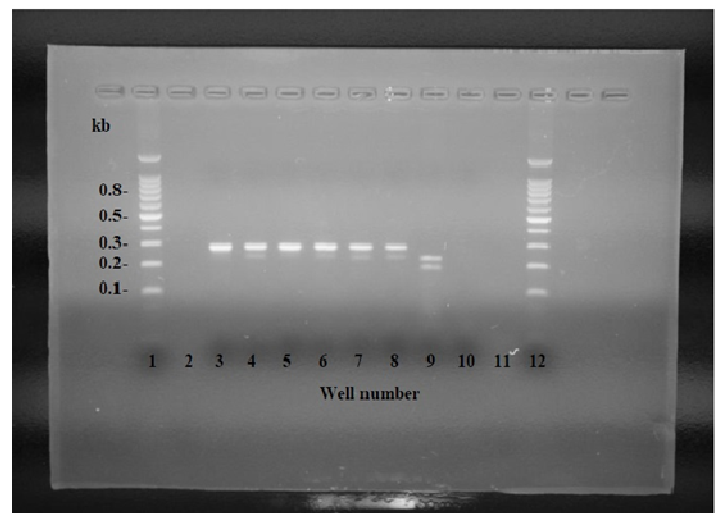
Figure 3. Qiagen DNAeasy PCR assay gel electrophoresis results

Discussion

A principal finding of the present study is that the MRSA incidence of 4.8% among the volunteers investigated is higher than the reported national incidence of 0.017% (37), but compares with a previous study conducted on New Zealand health workers in which 4% of samples tested were MRSA positive (38). While females were over-represented in all except the hospital patient groups, no significant difference in isolation rates was observed between the sexes.

Culture vs. PCR

The Roche PCR method was significantly more sensitive than the chromogenic agar method, detecting 2.2% more volunteers colonised with MRSA. In the current study, the PCR assay had a sensitivity of 100%, a figure previously reported by Boyce and Havill (13) but marginally higher than that reported in other studies in which nasal swab specimens were directly inoculated onto agar (range 90% to 98.5%; ref. 39-43) and similar to or better than in four other studies in which agar based cultures included a broth enrichment stage (range, 81% to 92%; ref. 41,43-45).

The addition of a broth enrichment step in the current study increased sensitivity by 1.1%. Figures ranging from 2% to 23% have been reported previously (41,43,45,46). Our study validates the usefulness of broth enrichment for enhancing sensitivity when using culture-based methods for detecting MRSA in swabs and the importance of this step in establishing a reliable gold standard in test evaluation studies (40). However, the addition of broth enrichment involves more personnel time and an added one to three days before results are available (13,44,47).

The specificity and PPV of the Roche LightCycler MRSA assay in the present study were 98.9% and 62.5%, respectively. Numerous studies have reported PPVs ranging from 63% to 95.8% which most likely reflects the differences in MRSA prevalence in the respective study populations (13,48). High sensitivity of any MRSA test is necessary because the objective of a MRSA screening program is to swiftly identify all those colonised with this bacterium, even if detection results from identifying DNA no longer associated with viable organisms (8). However, in a population with a modest level of asymptomatic MRSA colonisation, high test specificity can be important when undertaking a large number of tests to minimise false positive test results which can lead to inappropriate patient isolation (8).

Possible explanations for the observed differences in the detection rates between the culture method and the PCR assay could be: i) false positive PCR tests due to *mec*A loss from SCC*mec* cassettes, ii) MRSA being below the limit of detection of culture and/or the presence of substances in the specimen, such as antibiotics, which could inhibit the growth of MRSA but not interfere with PCR amplification of the organism and, iii) the ability of the PCR method to detect dead organisms. While false positive PCR tests that occur in the presence of "empty cassettes" resulting from the specific loss of *mec*A have been described previously (32,49,50), it is not something that happens very often (F. Merien, personal communication, March 17, 2012). However, this was a limitation in the present study that we can not investigate further. A possible solution would have been to use non-selective agar to allow growth of MSSA to examine the likelihood of this organism having a residual SCC*mec* right-extremity fragment following the deletion of a chromosomal segment containing *mec*A (32).

A previous study by Malhotra-Kumar et al. (15) found that chromogenic media tend to show reduced sensitivity at low MRSA bacterial loads. They concluded that this is due to some MRSA strains harbouring the SCC*mec* types III or IV which did not yield characteristic colonies at 24 h of incubation at MRSA loads of 1 and 10 colony forming units, or that the colony colour was not specific enough, causing some investigators to misinterpret these samples as MRSA negative. These conclusions may explain the inconsistency in medium performance seen between studies, which may be influenced by both, the predominant circulating MRSA types and by the differences in their colonisation potential (51).

Another major difference between the MRSA detection rates of PCR and culture is the ability of the latter to detect dead bacteria. PCR methods are able to detect organisms as long as the target DNA sequence is not injured, no matter whether the cell is viable, inactive or dead (52). Consequently, PCR techniques are evidently more sensitive than culture; nonetheless, they lack the ability to distinguish active cells from dead cells unless supplementary methods, such as viability assays, are used (53).

Interestingly enough, when we compared the isolation rate of MRSA from the nares and throat, via PCR and culture, the most noticeable increase in detection rate was from the anterior nares when using the PCR assay. PCR more than doubled the number of MRSA from the anterior nares when compared to culture (1.85% and 0.40%, respectively). This shows that even if PCR is applied to current MRSA screening practices (nasal only screening) current chromogenic methods may be missing

Test result turnaround time and cost
In the present study, the personnel hands on time per sample for the culture and PCR method were comparable (1.20 and 1.65 min, respectively). The significant difference was the time to result for each method. By using the Roche LC PCR assay, laboratories have the ability to obtain results within two to three hours. Realistically, in a clinical setting, where batch processing of samples for PCR assays is performed once a day, the turnaround time for the PCR method may equate to 10-15 hours, a figure previously reported by Boyce and Havill (13). Even if this was the case, the PCR method is still much faster. This allows earlier isolation and treatment of MRSA affected patients and hence is critical for the prevention of MRSA outbreaks. A study by Cunningham et al. demonstrated a reduction in the transmission of MRSA incidence from 13.9/1,000 patient days (under culture method) to 4.9/1,000 patient days under PCR screening (10). Additionally, the PCR method used in this study required only four hours of staff training with minimal expertise required to perform the assay.

One other major factor that all health institutions consider when implementing a new method is the cost of the test. Varying costs for chromogenic media and molecular methods have been previously reported: $7.52 and $25.50 per test, respectively (13), and € 40 versus € 4 per test, respectively (54). From our study, we have estimated that the Roche PCR assay costs 2.5 times more that the BBL CHROMagar method (data not shown). However, we believe that this additional cost may be offset by cost reductions due to pre-emptive isolation of patients. The objective of pre-emptive isolation is to prevent secondary MRSA cases while waiting for screening results. Specifically, this comes down to result turnaround times and test sensitivity, features that favour PCR methods over chromogenic methods. On the other hand, the cost efficacy of the direct PCR depends on the local infection control algorithms employed and the local epidemiology of MRSA. In patient populations with low MRSA incidence, the broad use of PCR probably is not cost-effective (55). As such, in countries like New Zealand where a MRSA incidence is modest, further local studies are required to assess the cost saving by employing a PCR method to replace or be run in parallel to current culture methods.

Transport media and organism viability
The present study suggests that the ESwab had a superior ability to maintain MRSA viability when compared to the Venturi counterpart. Additionally, the ESwab did have other subjective advantages. Being flocked, it was an easier swab system to use for collection as it allowed quicker uptake/absorption of surface mucous. Volunteers did not find this discomforting in comparison to the Dacron spun Venturi swabs. In fact, some volunteers found the ESwab more pleasant than the Venturi counterpart.

In the laboratory, the ESwab was much easier to manipulate than the Venturi swab due to the liquid media which allows the ability for multiple tests to be performed by using aliquots of the liquid media. Another advantage of the ESwab is its small size, flat base of the collection container, and the screw top cap which made the handling of the ESwab specimens much easier. The Venturi swab systems are long, have no screw-cap, and have a rounded base which makes them impossible to stand alone therefore one is forced to use both hands or a rack. The liquid phase is also an ideal platform for automated handling and specimen pooling. Two previous studies evaluated pooled against separate specimens for the detection of *S. aureus*. They concluded that pooling culture results for swabs from nares and the throat may be an appropriate method to optimise the yield of *S. aureus*–positive while saving the expenses of additional cultures (27,56). While the ESwab (NZ$1.39) is more expensive than the traditional Venturi swab (NZ$0.61), the additional cost may be offset by ability to pool samples.

Site specific MRSA colonisation

To our knowledge, this is the first study to have evaluated the importance of the throat in MRSA carriage in New Zealand. Previous studies have found a variable rate of *S. aureus* throat colonisation fluctuating from 4% up to 64% (25-29). In our study, MRSA was found in the throats of 61.5% (PCR) to 85.6% (culture) of all the MRSA positive volunteers. In 38.5% (PCR) to 42.8% (culture) of these volunteers, the throat was the only site from where MRSA could be isolated suggesting that individuals can be colonised exclusively in the throat and would be missed on screening limited to the anterior nares. Similar findings have been reported previously in studies from the USA and Australia (26,57).

The addition of the throat swab increased the overall MRSA yield from 3% (anterior nares only swab sampling) to 4.8% (anterior nares and throat swab sampling). We believe that unidentified throat carriers may spread MRSA. Mertz et al. (27) suggests that these unidentified MRSA throat carriers may explain, in part, why many decolonisation schemes are prone to failure. During our study, we found one hospital patient who had MRSA in the throat only who would have been missed with routine nasal screening. Certainly, throat carriage has been documented to cause MRSA outbreaks. Kluytmans et al. (58) documented a large outbreak of MRSA infection, which was traced back to a health care worker who was exclusively colonised in the throat. Routine nasal screening failed to identify this carrier.

Throat swab specimens have been obtained routinely in the Netherlands for decades as part of the successful search-and-destroy policy (27). Like Mertz el al. (27), we also think that an additional throat screening is very important during the investigation of MRSA carriers. Marshall and Spelman (26) suggest either throat or nose swabs are essential for MRSA detection, but both are preferable. We disagree with this conclusion as both sites are important. Screening of the anterior nares only should be substituted with screening of the nares and the throat and the costs of cultures of the nose and throat can be minimised by pooling the samples in the laboratory, which is applicable for both conventional cultures and PCR techniques (56).

Strain variation

As expected, all of the MRSA isolated during the study were penicillin resistant. This is because *mec*A encodes PBP2a which is not inhibited by ß-lactams. In the present study, the WSPP strain was the most frequently isolated strain, followed equally by the AK3 and WK/AK1 strains. While isolated in low numbers, our results are consistent with a previous report (59). However, it seems that the dominance of the WSPP clone in New Zealand is diminishing. Richardson and colleagues have documented that the prevalence of AK3 MRSA has increased each year since 2005, and in 2010 this strain accounted for the highest proportion (29.0%) of MRSA isolations (37). All the MRSA strains isolated in this study had type IV SCC*mec* elements. In New Zealand, it is now apparent that type IV SCC*mec* strains dominate MRSA isolations, and that this has been the case since 1995 (59). Since then, these strains have comprised on average almost three-quarters of all isolates per year, with a mean of 74% (37,59).

Although WPP, AK3, and WR/AK1 MRSA strains are considered community-associated, in our study they were all isolated from laboratory health workers and a hospital patient. We did not categorise volunteers according to where they acquired MRSA. Therefore, some participants who may have acquired MRSA in the community were categorised as hospital patients or staff. This is supported by the fact that the strains isolate in this study were not multi-resistant, a feature typical of community-associated MRSA (37). Additionally, as MRSA strains become progressively common in a variety of settings, the use of restrictive terms like HA-MRSA and CA-MRSA will become less meaningful as spread between all populations occur.

The potential to recover an increasing array of SCC*mec* types, especially from community-occurring MRSA strains, clearly exists (60).

Our study has some limitations. Firstly, by using clinical samples, we did not know the initial inoculum density therefore, the recovery percentage of the ESwab/conventional swab systems was not determined. Secondly, we did not include swabs utilised as zero-time controls. This was not possible for this real practice study as swabs would not be processed immediately after being taken. Thirdly, previous studies have focused on the CLSI M40-A protocol using a high initial inoculum for testing the swab systems. In our study, we tested the swab systems with clinical samples and therefore probably also with low numbers of microorganisms. Because of this, the release capability of the ESwab system was impossible to evaluate. Lastly, since we did not use non-selective agar, we were unable to determine if the PCR positive/culture negative results were due to methicillin-susceptible *S. aureus* isolates with a residual SCC*mec* right-extremity fragment following the deletion of a chromosomal segment containing *mecA* (32).

Conclusions

Our results with the Roche LightCycler MRSA Advanced Test show that it is an accurate and rapid method to detect MRSA colonisation, especially when compared to chromogenic culture. While the PCR method was more costly, it had superior sensitivity and specificity and was comparable to the culture method in regards to the amount of personnel hands on time per sample. The PCR assay can be performed by any microbiology personnel with minimal additional training and allows same day results. We therefore conclude that it wise to introduce PCR for MRSA screening.

Much effort has been expended to detect nasal MRSA carriers; however, throat carriers may contribute to spread MRSA infections. Our results support what has been previously reported; sampling from the anterior nares is insufficient for efficient detection of MRSA carriers. This study showed that the throat is an important habitat of MRSA hence any screening program for MRSA should include swab specimens from both, the anterior nares and the throat.

Both Copan swab types performed almost equally in maintaining the viability of MRSA during the study. While the ESwab was approximately double the price of the Venturi counterpart, it had a few advantages over the Venturi swabs system. The liquid phase allowed the pooling of sample and multiple testing to be performed from one swab specimen. Further studies are needed to determine if the higher cost of the ESwab will be offset by these advantages.

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