

Comparing a real-time multiplexed tandem PCR to the current culture-based method for detection of patients colonised with multi-drug resistant organisms.

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Background

Screening for multi-resistant organisms is an important but time-consuming activity for the Microbiology laboratory. During 2008, 16628 samples were screened for methicillin-resistant *S. aureus* (MRSA), 28325 for extended-spectrum beta-lactamase producing *Enterobacteriaceae* (ESBL) and 21027 for vancomycin-resistant Enterococci (VRE) at the Middlemore Hospital Laboratory. 3.6% of these screening samples were positive for MRSA, 4.8% positive for an ESBL and 0.2% positive for VRE. Large outbreaks due to ESBL have occurred at Middlemore Hospital in 2007-9, with smaller episodes of VRE and MRSA cross-transmission occurring in 2008. Regionally, VRE and ESBL outbreaks have occurred at Auckland City and North Shore Hospitals respectively in recent years.

Our current Infection Control policy for MRO screening states that all patients who have been in a hospital or long-term-care facility within the past 6 months are screened for MRO on each admission to a Counties Manukau District Health Board (CMDHB) facility. Adults who have been in a New Zealand facility are screened for MRSA, ESBL and VRE colonisation whereas children are currently only screened for MRSA. All patients who have been in an overseas facility are screened for carbapenem-resistant *Acinetobacter* in addition to MRSA, ESBL and VRE. A MRSA screen consists of sampling the nose, perineum, any non-intact skin e.g. chronic ulcers, surgical wounds and a catheter-urine sample from patients with a long-term indwelling urinary catheter. A rectal swab or faeces sample and midstream urine is used for screening for ESBL. The same rectal swab or faeces sample is also used for VRE screening. When resistant *Acinetobacter* screening is required, the samples collected for MRSA and ESBL/VRE screening are also used to screen for *Acinetobacter* colonisation.

If a patient has previously been found to be colonised with MRSA, ESBL or VRE during an admission to a CMDHB facility they have a warning attached to their national health index (NHI) number. Patients with such a NHI warning are not required to have repeat screening for that particular MRO but are screened for the other MROs. The NHI warning is visible in the patient information system (Figure 1).

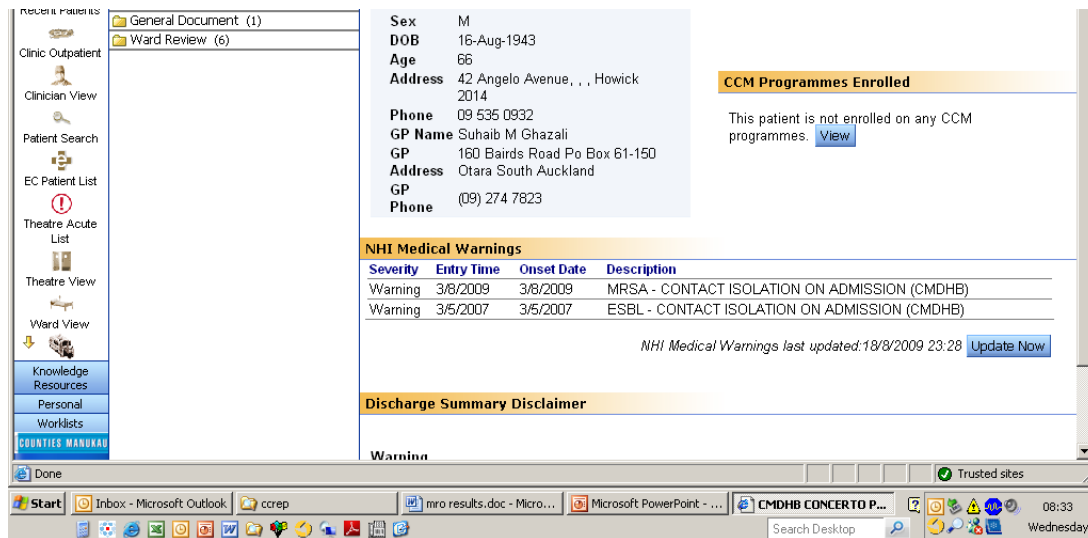


Figure 1. Example of MRSA and ESBL NHI warnings in Concerto.

Currently, patients who have been infected or colonised with an ESBL or VRE are assumed to remain colonised on future admissions regardless of subsequent negative screening results. In contrast, eradication of MRSA colonisation is sometimes attempted and patients may be removed from contact precautions in certain circumstances.

Patients who have been in an overseas hospital or who have a NHI MRO warning are placed in contact precautions on admission. Contact precautions include placement in a single room when possible. If there are insufficient single rooms available, patients with the same MRO are cohorted together in 2- or 4-bedded rooms. All other patients being screened for MRO are looked after using standard precautions unless their MRO screen returns positive, in which case they are moved into contact precautions.

Commercial assays that detect a single MRO by PCR, e.g. MRSA or VRE are available. Since CMDHB patients are screened for a panel of MROs, we have previously not seen an advantage for knowing individual MRSA or VRE results quickly, when decisions around patient placement or level of infection control precautions may be waiting on the ESBL culture result. An assay is now available that can detect multiple resistance genes from the same sample. The aim of this study was to determine whether a molecular method offered a benefit in detection or turn-around time compared with the conventional culture method.

Method

1. MRSA culture

180 samples received for MRSA screening were cultured by the current method used at Middlemore Hospital laboratory. Pairs of nasal and perineum swabs are processed as one sample by inoculating the nasal swab directly onto ½ of a chromID MRSA agar plate (bioMérieux). The nasal and perineum swab are then placed into the same tryptone mannitol salt broth (Fort Richard) and incubated at 37°C in air. The next day, the salt broth is subcultured onto ¼ chromID MRSA agar. The directly inoculated

chromID plate is examined for growth on day 1 and day 2. The broth inoculated chromID plate is examined on day 2. Any green colonies growing on the direct or broth-enriched chromID plates that agglutinate with StaphAurex Plus reagent (Oxoid) have susceptibility testing performed using the Vitek 2 AST-P579 card (bioMérieux). A test for staphylococcal thermostable nuclease is performed and if the patient has never had MRSA isolated before, a tube coagulase is also performed on the suspect colony. Any nuclease and/or coagulase positive isolates that are resistant to oxacillin are reported into the laboratory information system (LIS) as MRSA.

2. ESBL and VRE culture

180 samples received for ESBL and VRE screening were cultured by the current method used at Middlemore Hospital laboratory. A rectal swab or faeces sample is cultured directly on to ½ of a chromID ESBL agar plate (bioMérieux). The swab or a portion of faeces is then added to a bile-esculin broth containing 6mg/L vancomycin (Fort Richard). The ESBL agar is read after one day's incubation if the plate was inoculated before 2 pm and again on day 2 if the plate was inoculated after 2 pm. Any coloured colonies and oxidase-negative white colonies have an ESBL confirmatory test performed using 3 pairs of combination discs, ie ceftazidime, cefotaxime and cefepime with and without clavulanic acid (Bio-rad). Any *Enterobacteriaceae* were considered ESBL positive if the zone diameter around the cefotaxime disc with clavulanic acid and/or ceftazidime disc with clavulanic acid was ≥ 5 mm larger than the zone diameter around the corresponding disc without clavulanate or if the zone diameter around the cefepime and clavulanic acid was ≥ 4 mm larger than the zone around the cefepime disc without clavulanic acid. Pink colonies that were indole positive were reported as ESBL-producing *E. coli*, other fermentative isolates that were ESBL producers were identified to species using the Vitek 2 GN card (bioMérieux).

The bile-esculin broth was incubated in air at 37°C for 2 days. If the broth turned cloudy and black it was subcultured onto a ¼ plate of chromID VRE agar (bioMérieux). The VRE agar was incubated for 2 days and examined for blue or purple colonies. If these were present, the isolate was subcultured on to blood agar and identification performed using PYR, MADGE broth and Vitek 2 GP card (bioMérieux). Susceptibility testing was performed using the Vitek 2 AST-P579 card.

If the patient had been positive for an ESBL-producing *Enterobacteriaceae* in the past, any growth on chromogenic agar was not further worked up but reported with a comment stating that because the patient was previously positive, repeat testing was not indicated.

3. Detection of resistance genes by PCR

Between January and May 2009, 180 samples submitted for MRSA screening by culture and 180 samples submitted for ESBL and VRE screening by culture were selected for testing using the AusDiagnostics Robot, Rotor-gene and Easy-Plex Infection Control panel 3104.02, (Figure 2). This panel detects the following genes by a tandem multiplex PCR method: *nuc* (*S. aureus* thermostable nuclease gene), *mecA* (Methicillin resistance gene), SCC (Staphylococcal cassette chromosome), *vanA* (vancomycin resistance gene A), *vanB* (vancomycin resistance gene B), and the beta-

lactamase genes CTX-M group 1, CTX-M group 9. Six samples can be tested per batch and each batch takes 2 hours to complete.

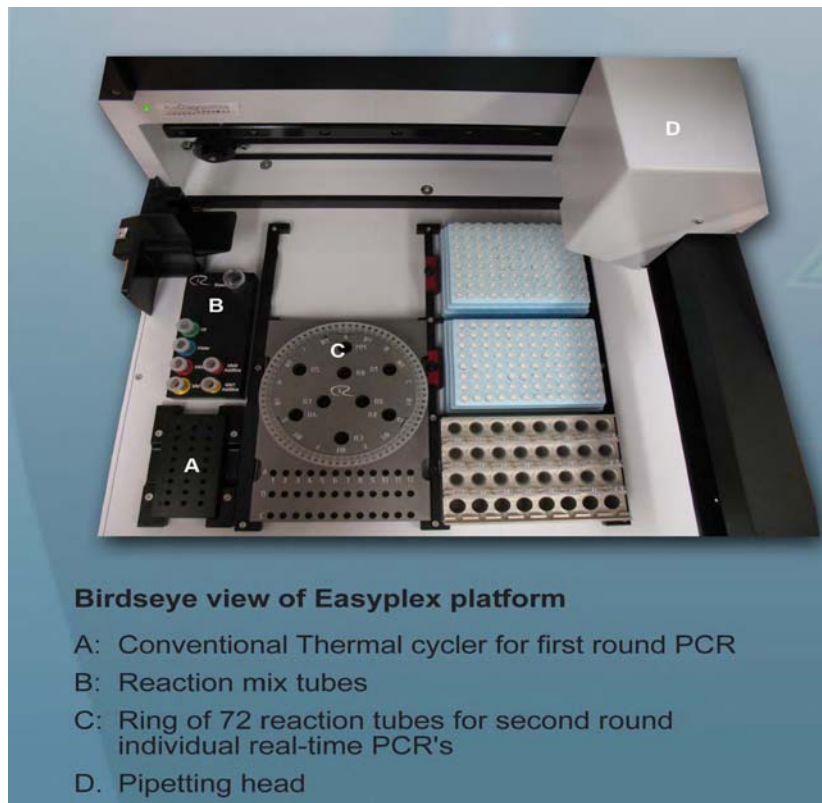


Figure 2. AusDiagnostics Robot.

In order to increase the chance of testing a patient colonised with an MRO the patient information system (Concerto) was reviewed for MRO warnings. We aimed to include at least one patient with a MRO warning in each batch of 6 samples.

If the Easy-Plex assay became a routine test, we did not anticipate being able to use it during the evening or post-midnight shifts. So rather than testing swabs directly, a sample from an overnight incubated broth was used. For the MRSA samples, the tryptone mannitol salt broth was sampled. For the ESBL/VRE samples, the rectal swab or a portion of faeces was inoculated into a MacConkey broth containing 2mg/L cefotaxime and incubated overnight. A 100 μ L sample of the broths was diluted in 900 μ L of saline and 10 μ L of this was used in the testing wells.

Mannitol salt broths that were positive for a beta-lactamase gene were subcultured onto ChromID ESBL agar (BioMérieux). MacConkey broths that were positive for CTX-M genes but negative on the direct ESBL culture were also subcultured on to ChromID ESBL agar.

Dates and times were recorded for the patient's admission, the registration of the sample in the laboratory, the culture result and the PCR result. The day the sample was collected was counted as day 0.

Sensitivity and specificity of PCR results to predict culture results and their 95% confidence intervals were calculated.

Results

1. MRSA screening samples

180 MRSA specimens from 165 patients were tested. The majority of the samples were combined nasal/perineum swabs (169). The remainder included wound swabs (9) and nasal swabs (2). Specimens mainly came from adults; there were 5 patients <16 year olds, 30 patients aged 16-45 years, 44 aged 46-65 years, 69 aged 66-85 years and 17 patients aged 86-99 years. Four screening samples were requested on outpatients. The remaining 176 screening samples were requested on inpatients. The inpatients being screened had been admitted to medical (90), surgical (50), rehabilitation (21), Women's Health (6), intensive care (4), and paediatric (5) wards.

50 patients had no NHI MRO warning and 115 had at least one MRO warning. 35 patients had a MRSA warning, 63 had an ESBL warning, 15 patients had warnings for both ESBL and MRSA, 1 patient had warnings for both MRSA and VRE and 1 patient had warnings for both ESBL and VRE.

138/176 (78.4%) of samples from inpatients were collected on days 0-2 of the admission, 38 samples were requested on or after day 3 of the patient's admission.

39 samples from 35 patients isolated MRSA. 32 of these samples were positive for *nuc*, *mecA* and SCC, 2 culture positive samples were positive for *mecA* and SCC, 2 were positive for *nuc* and *mecA* only. MRSA was isolated from 14 patients for the first time, in one case this would not have been detected by the Easy-plex assay. There were 2 samples that were positive for *nuc*, *mecA* and SCC but MRSA culture negative. (Table 1)

Table 1. Comparison of MRSA detection by culture and Easy-plex assay.

Easy-plex result	MRSA culture positive	MRSA culture negative
<i>nuc</i> , <i>mecA</i> and SCC detected	32	2
<i>mecA</i> and SCC detected	2	0
<i>nuc</i> and <i>mecA</i> detected	2	34
≤1 of <i>nuc</i> or <i>mecA</i> detected	3	105
Total	39	141

Table 2. Sensitivity, specificity, positive predictive value and negative predictive value of PCR testing results against MRSA culture results

	PCR test positive: All three genes are detected as positive	PCR test positive: At least two genes are detected as positive
Sensitivity	0.82 (95% CI: 0.66 -0.92)	0.92(95% CI: 0.79-0.98)
Specificity	0.97 (95% CI: 0.93-0.99)	0.74(95% CI: 0.66-0.81)
Positive predictive value	0.89 (95% CI: 0.74-0.97)	0.50(95% CI: 0.38-0.62)
Negative predictive value	0.95 (95% CI: 0.90 - 0.98)	0.97(95% CI: 0.92-0.99)

32 (18%) mannitol salt broths were retrospectively cultured onto chromID ESBL agar because of the presence of CTX-M group 1 and/or CTX-M group 9 genes. An ESBL producing *Enterobacteriaceae* was isolated from 29 samples. Most of these patients had an ESBL MRO warning already, but in 3 cases this was the first time an ESBL had been detected.

There were 3 samples that were positive for CTX-M group 9 yet an ESBL was not recovered from the mannitol salt broth. However, 2 of these patients were known to be colonised with an ESBL.

One mannitol salt broth was positive for *vanA* and 1 was positive for *vanB*. A vancomycin-resistant *Enterococcus* was isolated on retrospective culture from each of these mannitol salt broths.

Of the 141 negative culture results, 103 (73%) were reported in the LIS on day 2. The other 38 negative culture results were reported on day 3. Of the 39 screening samples that isolated MRSA, 2 samples were reported with a probable MRSA on day 1, 24 results were reported with confirmed MRSA on day 2, 10 on day 3 and 2 on day 4.

Easy-plex results were all available on day 1. There were 121 samples from patients who did not have a MRSA NHI warning, 51 from patients with no MRO warning and 70 from patients with an ESBL and/or VRE warning. If the Easy-plex assay was used to decide which broths needed to be subcultured, 85/121 (70%) of the mannitol salt broths could be reported as negative for MRSA on day 1 based on having ≤ 1 out of 3 of *nuc*, *mecA* and SCC genes compared with 58 results being reported culture negative on day 2 and 27 results being reported culture negative on day 3. 14 (12%) samples could be reported as presumptively positive for MRSA based on the presence of *nuc*, *mecA* and SCC on day 1. If the presence of *nuc* and *mecA* without detectable SCC in a salt broth was interpreted as possibly containing MRSA then 22/121 of the mannitol salt broths would need further subculture.

2. ESBL and VRE screening samples

180 specimens from 171 patients were tested. The majority of the samples were rectal swabs (155). The remainder were faeces (20) and wound swabs (5). Due to the MRO screening policy, specimens mainly came from adults. There were 2 patients <16 years, 25 patients aged 16-45 years, 37 patients aged 46-65 years, 82 patients aged 66-85 years and 25 patients aged 86-99 years. 19 specimens came from outpatients; 6 were on dialysis, 11 lived in rest homes and 2 were attending pre-admission clinics. The 161 specimens from inpatients came from patients admitted to medical (81), surgical (45), rehabilitation (26), Women's Health (6), intensive care (1) and paediatric (2) wards.

97 patients had no NHI MRO warning and 74 patients had at least one MRO warning. 16 patients had a MRSA warning, 51 patients had an ESBL warning, 1 patient had a VRE warning, 5 patients had warnings for both MRSA and ESBL and 1 patient had warnings for MRSA, ESBL and VRE.

116/161 (72%) of samples from inpatients were collected on days 0-2 of the admission, 45 samples were requested on or after day 3 of the patient's admission.

12 samples isolated ESBL. 10 of these samples were positive for the CTX-M group 1 gene and 2 samples were positive for CTX-M group 9 gene. There were also 27 specimens from 26 patients with growth on the ChromID ESBL agar that was not further worked up because of a past history of ESBL colonisation. 20 of these samples were positive for CTX-M group 1 and 1 was positive for CTX-M group 9. 141 samples from 136 patients were culture negative using the routine method for ESBL detection. 2 out of these 141 samples were positive for CTX-M group 1 and ESBLs were isolated from the MacConkey cefotaxime broth when subcultured. One sample was positive for CTX-M group 9, but no ESBL was detected when the MacConkey cefotaxime broth was subcultured on to chromID agar.

Of the 141 negative culture results, 44 (31%) were reported in the LIS on day 1, 91 (65%) were reported on day 2, 5 were reported on day 3 and for 2 results the time of reporting was not available. For the 12 samples that isolated an ESBL, 7 were reported on day 2 and 5 were reported on day 3.

Easy-plex results were all available on day 1. There were 115 samples from patients who did not have an ESBL NHI warning. If the Easy-plex assay was used to decide which broths needed to be subcultured, 107/115 (93%) of the cefotaxime MacConkey broths could be reported as negative for ESBL genes on day 1 compared with 74 results being reported culture negative on day 2 and 4 results being reported culture negative on day 3. Seven samples could be reported positive for an ESBL gene on day 1 which was 1 day earlier than culture for 3 samples and 2 days earlier for 4 samples.

There was only one sample from a patient previously colonised with VRE. Vancomycin resistance genes were not detected from this sample. No other samples isolated VRE or detected *vanA* or *vanB*. Negative VRE culture results were reported on day 2 for 39% of samples, day 3 for 21%, day 4 for 26%, day 5 for 12% and day 6 for 2%.

Discussion

The AusDiagnostic assay was more sensitive than culture for the detection of ESBL producing *Enterobacteriaceae*. For the ESBL screening samples more than 70% of negative results could be reported at least one day earlier compared with culture.

The ability of this version of Easy-Plex assay to detect MRSA genes was less optimal. If the presence of SCC, *mecA* +/- *nuc* was considered a presumptive MRSA result the sensitivity was 87% and specificity 98% compared with culture. If the presence of any 2 MRSA genes was considered a possible MRSA result the sensitivity was 92% and specificity 74% compared with culture.

If the Easy-plex assay was introduced as a routine test, it would be reserved for patients who did not already have a MRO warning since there would be little advantage in a more expensive yet rapid negative result from a patient known to have a history of MRO colonisation. Pre-selection of samples would also be required since the number of samples received each day for testing (~65 MRSA and 110 ESBL screens during 2009) exceeds the capacity of one AusDiagnostic unit. Only 4 batches could be run during the 8 hour day shift unless testing also occurred in the evening shift or more than one unit was available for testing. Keeping with a 12-gene profile would also limit batches to 6 samples. If fewer resistance genes were tested, e.g. only the ESBL and VRE genes, then 48 samples could be processed during one shift. It may also be possible to combine rectal swab and urine samples from the same patient and process as a single test, but this approach would need further validation before introducing.

Given that ESBL cross-transmission in CMDHB facilities has been a greater problem in recent years than MRSA, it is recommended that the AusDiagnostic assay be used to provide faster ESBL results for patients without ESBL NHI warnings. The version of MRSA genes used during the trial was not sensitive enough for this to be used at this time. However, newer version of MRSA assays could be re-evaluated in the future. Periodic surveillance to ensure that the CTX-M group 1 and 9 remain the predominant ESBL genes locally would be required.

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