Maisie Cao[1], John T. Ellis[2], Deborah Marriott[1], John Harkness[1], Damien Stark[1]

[1]Division of Microbiology, Sydpath, St Vincent's Hospital, Darlinghurst, NSW, Australia; [2]School of Life Sciences, University of Technology Sydney, Broadway, NSW, Australia

Contact Maisie Cao.
E-mail: maisie.cao@svha.org.au

1. Cooper CJ, Fleming R, Boman DA, Zuckerman MJ. Varied clinical manifestations of amebic colitis. *South Med J* 2015; 108: 676–81.
2. Stark D, van Hal S, Fotedar R, *et al*. Comparison of stool antigen detection kits to PCR for diagnosis of amebiasis. *J Clin Microbiol* 2008; 46: 1678–81.
3. Stark D, Roberts T, Ellis JT, Marriott D, Harkness J. Evaluation of the EasyScreen enteric parasite detection kit for the detection of Blastocystis spp., Cryptosporidium spp., Dientamoeba fragilis, Entamoeba complex, and Giardia intestinalis from clinical stool samples. *Diagn Microbiol Infect Dis* 2014; 78: 149–52.
4. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J. Laboratory diagnostic techniques for Entamoeba species. *Clin Microbiol Rev* 2007; 20: 511–32.
5. Stark DJ, Fotedar R, Ellis JT, Harkness JL. Locally acquired infection with Entamoeba histolytica in men who have sex with men in Australia. *Med J Aust* 2006; 185: 417.
6. Fotedar R, Stark D, Marriott D, Ellis J, Harkness J. Entamoeba moshkovskii infections in Sydney, Australia. *Eur J Clin Microbiol Infect Dis* 2008; 27: 133–7.
7. Stark D, Al-Qassab SE, Barratt JL, *et al*. Evaluation of multiplex tandem real-time PCR for detection of Cryptosporidium spp., Dientamoeba fragilis, Entamoeba histolytica, and Giardia intestinalis in clinical stool samples. *J Clin Microbiol* 2011; 49: 257–62.
8. Stark D, Beebe N, Marriott D, Ellis J, Harkness J. Detection of Dientamoeba fragilis in fresh stool specimens using PCR. *Int J Parasitol* 2005; 35: 57–62.
9. Ahmad N, Khan M, Hoque MI, Haque R, Mondol D. Detection of Entamoeba histolytica DNA from liver abscess aspirate using polymerase chain reaction (PCR): a diagnostic tool for amoebic liver abscess. *Bangladesh Med Res Counc Bull* 2007; 33: 13–20.
10. Jaiswal V, Ghoshal U, Baijal SS, Mittal B, Dhole TN, Ghoshal UC. Evaluation of antigen detection and polymerase chain reaction for diagnosis of amoebic liver abscess in patients on anti-amoebic treatment. *BMC Res Notes* 2012; 5: 416.
11. McAuliffe GN, Anderson TP, Stevens M, *et al*. Systematic application of multiplex PCR enhances the detection of bacteria, parasites, and viruses in stool samples. *J Infect* 2013; 67: 122–9.
12. Stark D, Barratt J, Roberts T, Marriott D, Harkness J, Ellis J. Comparison of microscopy, two xenic culture techniques, conventional and real-time PCR for the detection of Dientamoeba fragilis in clinical stool samples. *Eur J Clin Microbiol Infect Dis* 2010; 29: 411–6.

DOI: https://doi.org/10.1016/j.pathol.2019.01.012

Increasing prevalence of methicillin-resistant *Staphylococcus aureus* in remote Australian communities: implications for patients and clinicians

Sir,

Staphylococcus aureus is a leading cause of life-threatening community and hospital-acquired infection. Methicillin-resistant *S. aureus* (MRSA) was initially limited to the hospital environment [healthcare-associated MRSA (HA-MRSA)]; however, in Australia, community-acquired MRSA (CA-MRSA) is now commonly isolated, particularly in Indigenous Australians.[1,2] In the Northern Territory, Western Australia and New South Wales, residence in a remote setting is also an independent predictor of CA-MRSA isolation.[1–3] This has important implications for empirical antibiotic regimens in these locations, where clinicians usually have limited access to microbiology laboratory services and are often a long way from sophisticated critical care support.

Far North Queensland (FNQ) covers an area of 380,000 km^2 in tropical Australia and has a population of approximately 280,000 people, 12% of whom identify as Indigenous Australians. The AUSLAB database records the pathology results of all the hospitals and community clinics in Queensland's public health system. To determine the changing local antibiotic susceptibility of *S. aureus*, AUSLAB was interrogated to identify all clinical isolates collected in FNQ between 1 January 1997 and 31 December 2016 and their patterns of antibiotic resistance. In the absence of genetic testing, MRSA was defined as *in vitro* resistance to flucloxacillin; while CA-MRSA was defined as *in vitro* resistance to flucloxacillin but susceptibility to non-beta-lactam antibiotics.[4] The geographical location of each isolate and basic demographic data including patient age, gender, residential address and Indigenous status were recorded. Groups were compared using the chi-squared test; logistic regression analysis was performed using statistical software (Stata version 14.2; StataCorp, USA). Maps were generated using geographic information system software (MapInfo Pro version 15.0; Pitney Bowes, USA) with FNQ divided into eight areas based on key clinical hubs. The Far North Queensland Human Research Ethics Committee provided ethical approval for the study (HREC/16/QCH/112–1085) and waived the requirement for informed consent as the data were retrospective and de-identified.

After excluding non-FNQ residents and repeated isolates from the same patient within a 12-month period, *S. aureus* was isolated on 46,304 separate occasions; 36,802 (79%) were methicillin-sensitive, 8766 (19%) were MRSA, while in 736 (2%) incomplete resistance data precluded classification. Of the 8766 MRSA isolates, 8038 (92%) had antibiograms consistent with CA-MRSA. There was an increase in the prevalence of MRSA over the study period, from 187/786 (24%) in 1997 to 1388/4373 (32%) in 2016 (p for trend <0.001). In some regions this was particularly notable: in the area around Cooktown, MRSA isolates increased from 2/5 (40%) [95% confidence interval (CI) 5–85%] in 1997 to 24/33 (73%) in 2016 (p for trend <0.001) (95% CI 55–87%) (Fig 1). In 2016, 68/3729 (2%) *S. aureus* isolates were resistant to sulfamethoxazole-trimethoprim; inducible resistance to clindamycin was reported in 368/4350 (9%). There were no cases of vancomycin resistance during the study period.

In univariate analysis, MRSA was more commonly isolated in Indigenous patients than non-Indigenous patients [odds ratio (OR) 1.59; 95% CI 1.51–1.68; $p<0.001$]; in patients aged $\geq$40 than aged <40 years (OR 1.19; 95% CI 1.12–1.24; $p<0.001$); and in patients living in metropolitan Cairns than those from a remote setting (OR 1.28; 95% CI 1.22–1.34; $p<0.001$). There was no difference in univariate analysis between the prevalence among men and women [5296/25972 (21%) versus 4170/20295 (21%), p=0.97; in 217 cases the patient's gender was not available].

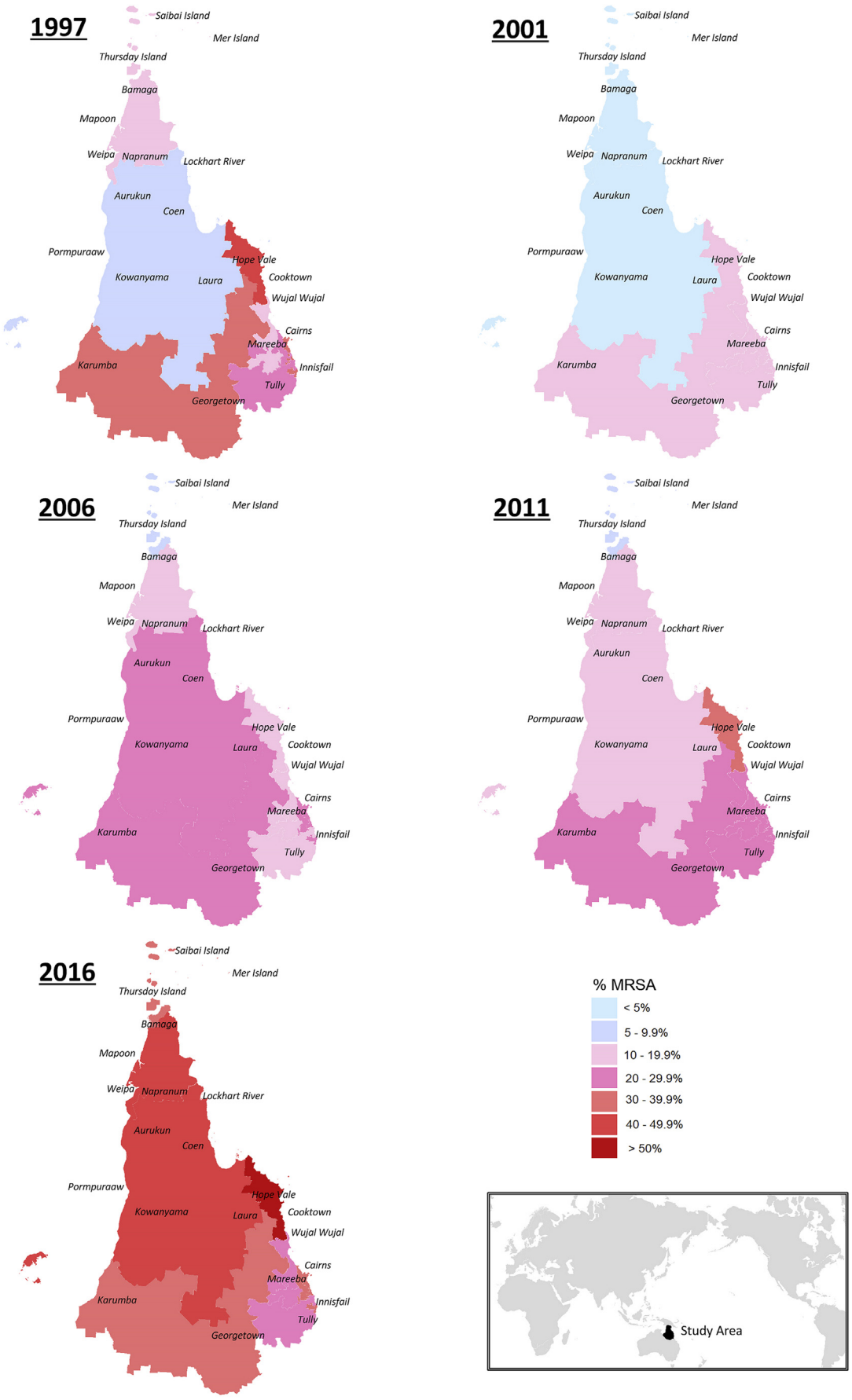

Fig. 1 Temporospatial epidemiology of methicillin-resistant *Staphylococcus aureus*; presented as a proportion of all *S. aureus* isolates.

In multivariate analysis, MRSA was more common in Indigenous patients than non-Indigenous patients (OR 1.79; 95% CI 1.70−1.90; $p<0.001$) and in patients living in metropolitan Cairns than those from a remote area (OR 1.46; 95% CI 1.38−1.55; $p<0.001$). In multivariate analysis, age was no longer an independent predictor of MRSA status, but gender was, with MRSA occurring more commonly in men (OR 1.06; 95% CI 1.01−1.12; $p=0.02$).

While the national prevalence of MRSA amongst all *S. aureus* isolated in Australia has remained relatively stable at 10−12% over the past 10 years,[4] the prevalence in FNQ is presently almost three times this and is continuing to rise. Meanwhile, the current prevalence of MRSA of 73% in the Cooktown region in this study is the highest ever reported in Australia.

This increasing prevalence of MRSA is seen in other remote regions around Australia. Across the Northern Territory, the prevalence of MRSA increased from 7% to 24% between 1993 and 2012.[5] A 2017 study examining purulent skin and soft tissues infections around Alice Springs was the first to report MRSA superseding methicillin-susceptible *S. aureus* (MSSA) as a cause of community onset staphylococcal infections, representing 60% of all isolates.[6] A similar pattern is seen in New South Wales and Western Australia, with the prevalence and incidence of MRSA higher in remote locations (58% and 3546 per 100,000, respectively).[1,7]

The strongest recorded risk factor for MRSA isolation in this series was an Indigenous background, echoing other Australian studies that have identified a higher rate of MRSA in Indigenous individuals.[1,2] This association between MRSA and Indigenous status has been strongly linked to the marked socioeconomic disadvantage that is seen in many Indigenous communities.[8] Factors hypothesised to facilitate MRSA transmission in Indigenous populations include overcrowding, higher rates of staphylococcal colonisation, high rates of infective skin disease and, in some cases, unreliable water supply.[8] Greater exposure to β-lactam antibiotic therapy for recurrent skin and respiratory tract infection has also been hypothesised to increase selection pressure.[8]

It is notable that while a large series from the Northern Territory identified a higher prevalence in remote communities and in women, the converse was true in our series.[2] Meanwhile a study from Northern New South Wales reported a higher prevalence in the local Indigenous population, although it also noted that MRSA prevalence has actually been declining in recent years.[1] This heterogeneity across different populations suggests that while similar underlying trends may exist, there are a variety of social, behavioural and biological factors that impact on MRSA incidence at a local level.

It is unclear why MRSA prevalence was higher in males in our region, but it could be explained by higher rates of nasal carriage, differing hand-hygiene behaviour or increased involvement in contact sports.[9] The finding that MRSA was more common in metropolitan Cairns than in remote, rural communities, contrasts with several other studies that have found residence in a remote community to be a stronger predictor of MRSA carriage.[1,7] Potential explanations for this observation include the significant mobility of local populations as well as the disproportionate level of socioeconomic disadvantage found in many areas of metropolitan Cairns, which is significantly higher than the regional, state and national averages.[10]

However, it is important to note that while there are differences between our findings and those from other locations, there was even a marked local heterogeneity within this study's cohort, a further reminder that all microbiology is local.[11] While MRSA rates were high across the region, in 2016 the prevalence of MRSA varied from 20% around Innisfail to 73% around Cooktown. This variation is presumably due again to differences in socioeconomic factors, patterns of social interaction and antibiotic prescription across FNQ.[11]

These findings have major implications for the empirical antibiotic therapy of skin and soft tissue infections and sepsis in FNQ, particularly in remote settings where many patients are managed and where there is frequently limited laboratory support. In the patient presenting with sepsis without a source apparent, vancomycin should be added to empirical regimens. For skin and soft tissue infections, sulfamethoxazole-trimethoprim or clindamycin should be recommended as empirical oral therapy. Clearly, ongoing surveillance is necessary to identify evolving resistance.

Stemming the increasing prevalence of MRSA in remote settings has proven to be challenging. Infection control measures including hand hygiene are effective for reducing hospital-acquired MRSA but are more difficult to implement in the community setting. A focus on hand hygiene has the advantage of addressing other pathogens simultaneously, although other measures including case finding and eradication would be required in patients with recurrent infections. Simple hygiene messages should be reinforced, although as the infection appears to be a marker of socioeconomic disadvantage, public health policies that address this fact at a regional and national level are more likely to have an impact on both MRSA prevalence and health outcomes generally.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Isabel Guthridge[1], Simon Smith[2], Peter Horne[3], Josh Hanson[2,4]

[1]Cairns Clinical School, James Cook University, Cairns Hospital, Cairns, Qld, Australia; [2]Division of Medicine, Cairns Hospital, Cairns, Qld, Australia; [3]Health Surveillance, Tropical Public Health Services Cairns, Cairns, Qld, Australia; [4]The Kirby Institute, University of New South Wales, Kensington, NSW, Australia

Contact Isabel Guthridge.
E-mail: isabel.guthridge@my.jcu.edu.au

1. Agostino JW, Ferguson JK, Eastwood K, *et al*. The increasing importance of community-acquired methicillin-resistant Staphylococcus aureus infections. *Med J Aust* 2017; 207: 388−93.
2. Tong SYC, Bishop EJ, Lilliebridge RA, *et al*. Community-associated strains of methicillin-resistant Staphylococcus aureus and methicillin-susceptible S. aureus in Indigenous Northern Australia: epidemiology and outcomes. *J Infect Dis* 2009; 199: 1461−70.
3. Riley TV, Rouse IL. Methicillin-resistant Staphylococcus aureus in western Australia, 1983−1992. *J Hosp Infect* 1995; 29: 177−88.
4. Turnidge J, Coombs G, Daley D, *et al*., Australian Group on Antimicrobial Resistance (AGAR) participants, 2000−14. *MRSA: A Tale of Three Types. 15 Years of Survey Data From AGAR*. Sydney: ACSQHC, 2016.

5. Tong SYC, Varrone L, Chatfield MD, *et al*. Progressive increase in community-associated methicillin-resistant Staphylococcus aureus in Indigenous populations in northern Australia from 1993 to 2012. *Epidemiol Infect* 2014; 143: 1519–23.
6. Macmorran E, Harch S, Athan E, *et al*. The rise of methicillin resistant Staphylococcus aureus: now the dominant cause of skin and soft tissue infection in Central Australia. *Epidemiol Infect* 2017; 145: 2817–26.
7. Coombs G, Pearson J, Robinson O. Western Australian methicillin-resistant Staphylococcus aureus (MRSA) epidemiology and typing report: July 1 2016 to June 30 2017. Dec 2017; cited 6 Sep 2018. https://ww2.health.wa.gov.au/~/media/Files/Corporate/general%20documents/Infectious%20diseases/PDF/HISWA/Annual%20reports/WA_annual_report_MRSA_2016_2017.pdf
8. Turnidge JD. High burden of staphylococcal disease in Indigenous communities. *J Infect Dis* 2009; 199: 1416–8.
9. Humphreys H, Fitzpatick F, Harvey BJ. Gender differences in rates of carriage and bloodstream infection caused by methicillin-resistant Staphylococcus aureus: are they real, do they matter and why? *Clin Infect Dis* 2015; 61: 1708–14.
10. Cairns Regional Council. *Cairns SEIFA profile by area*. Cited 17 Jul 2018. https://profile.id.com.au/cairns/seifa-disadvantage-small-area
11. Tong SY, Chen LF, Fowler Jr VG. Colonization, pathogenicity, host susceptibility, and therapeutics for Staphylococcus aureus: what is the clinical relevance? *Semin Immunopathol* 2012; 34: 185–200.

DOI: https://doi.org/10.1016/j.pathol.2018.11.015

Candida auris arriving on our shores: an Australian microbiology laboratory's experience

Sir,

Nosocomial outbreaks of *Candida auris* have been reported globally, predominantly from India, the United States and the UK. Although first reported from Japan in 2009, a retrospective analysis discovered a misidentified isolate from a case of fungaemia from 1996 in South Korea.[1,2] The identification of *C. auris* is challenging for routine laboratories, particularly where matrix assisted laser desorption/ionisation–time of flight mass spectrometry (MALDI-TOF MS) technology is not available, as biochemical methods are often unable to differentiate *C. auris* from other closely related *Candida* species.

We wish to share our experience in the recent identification of *C. auris* isolated from a urine specimen. It was initially presumptively identified by MALDI-TOF MS (Bruker Daltonics, USA), and subsequently confirmed by sequencing of the internal transcribed spacer (ITS) regions of the ribosomal DNA. Additionally, Vitek 2 YST (software version 8.01) identified it as *C. auris* with 99% probability.

In July 2018, a urine specimen was submitted for microscopy, culture and sensitivities; no clinical information was provided on the request form. Cell count results were: leukocytes 2×10^6/L, erythrocytes 2×10^6/L, 0 epithelial cells; culture on Columbia HBA and MacConkey agar (aerobic, 35°C) had 10^9 CFU/L pure growth of yeast after 24 h. This was presumptively identified by the MALDI-TOF MS Biotyper 3.1 using the Research Use Only (RUO) database as *C. auris* with a log score of 1.75 by direct formic acid extraction, which improved to 1.86 when the ethanol formic acid extraction method was used. Three *C. auris* isolates are represented in the RUO database, and all appeared in the top three identifications with no other *Candida* spp. in the top ten scores.

The isolate was referred to the National Mycology Reference Centre (NMRC, Adelaide, SA) for confirmation of identification and susceptibility testing. MALDI-TOF MS Bruker identification using a supplemented database of known *C. auris* isolates (log score 1.85 by direct formic acid extraction), coupled with ITS sequencing (100% sequence identity to curated sequences from confirmed *C. auris* isolates; http://www.westerdijkinstitute.nl and http://www.fungalbarcoding.org), confirmed the identification as *C. auris*. The in-house Bruker database of the NMRC contains the spectra of three additional *C. auris* strains obtained from international laboratories and definitively identified by ITS sequencing. The ITS sequence obtained from this isolate was submitted to Genbank (accession no. MK367811).

A review of the literature showed the identification of *C. auris* isolates using the Bruker RUO library occasionally yielded log scores of $\leq$1.8 with the direct formic acid extraction method but improved to $\geq$2.0 using the full in tube extraction method.[3,4] When we performed the full ethanol formic acid extraction method the log score improved only to 1.86. This may be explained by the limited representation of *C. auris* spectra in the database in comparison to clinical isolates. Indeed, when the isolate was re-identified after a recent manufacturer update of the RUO database with six additional C. *auris* reference spectra, a log score match of $>$2.0 to all six new spectral profiles was obtained by the direct formic acid extraction method. A MALDI-TOF MS log score of $\geq$1.7 is sufficient for species level identification of common *Candida* spp. as reported in several validation studies.[5,6] There is less experience with the score threshold required for an acceptable identification of *C. auris,* but the absence of reports of other yeasts being misidentified as *C. auris* is reassuring in this respect. The TGA registered Bruker IVD library for clinical use now has the same nine strains of *C. auris* included in the database (MBT IVD Library DB-7712, April 2018). If required, CDC's RUO Bruker database which is accessible on MicrobeNet has four reference spectra of *C. auris*, one from each of the four phylogenetic clades.

For users of the Vitek MALDI-TOF MS system (bioMérieux, France), the routine clinical use database (Version 3.2.0) has been updated to include *C. auris* as of June 2018; its performance in the Australian clinical setting remains to be determined.

Vitek 2 YST (software version 8.01) identification was performed from day 1 and day 2 Sabouraud's dextrose agar (SAB) subcultures, both with identification of *C. auris* at 99% probability. Much of the literature warns of misidentifications of *C. auris* as *Candida haemulonii* complex by Vitek 2 YST, as *C. auris* is not represented in previous database versions. Almost all Vitek 2 YST users in Australia have now been upgraded to software version 8.01 with the inclusion of *C. auris* (personal correspondence, bioMérieux, Australia). Despite this improvement, any identification of *Candida haemulonii* or *Candida duobushaemulonii* by Vitek 2 YST must still prompt the exclusion of *C. auris* by a more reliable method of identification. With the exception of Vitek 2 YST, none of the other phenotypic identification systems have included *C. auris* in their databases to date, and therefore cannot be used to identify or exclude *C. auris*.

Additional phenotypic investigations were performed retrospectively. Macroscopically, colonies were smooth and white-cream on SAB, beige on Brilliance Candida Agar