Prior antifungal exposure is instrumental in driving resistance. In the face of cumulative clinical experience with the echinocandins, uniformly low MICs were observed for the non-*albicans* species, save one strain each of *C. glabrata* and *C. haemulonii.* This lends credence to our institutional antifungal policies, despite the absence of a formal antifungal stewardship program. The uniform susceptibility pattern observed may also be attributable in part to it being a predominantly geriatric centre, which does not share the vulnerable population of haematopoietic stem cell transplant patients where empirical and prophylactic usage is widely prevalent.

The epidemiology in North America is unlike that of the Asia-Pacific region, where alarming rates (~12%) of echinocandin resistance have been encountered in *C. glabrata.*[12] Many strains were simultaneously non-susceptible to the azoles, effectively rendering them multidrug resistant.

Formalisation of the stewardship program at our tertiary care centre would enable longitudinal monitoring of known risk factors for emerging resistance. The microbiology laboratory is in an ideal position to support this by continuing surveillance of susceptibility patterns. Although resistance to the echinocandins may eventually be inevitable, this class of antifungals currently holds promise as the mainstay for therapy of *Candida* BSI.

Our study adds to the growing knowledge on antifungal resistance in geriatric cohorts as the average life expectancy steadily rises across the developed world. A similar study in elderly patients from Italy documented uniform susceptibility among non-*albicans* species to caspofungin.[13] Close to home, in China, rates of non-susceptibility to echinocandins were nil from an independent geriatric cohort.[14] This study reaffirms the position of the laboratory as the sentinel site for detecting acquired antifungal resistance, long before clinical failure is documented. It also emphasises the developing role of antifungal stewardship across particularly vulnerable patient groups.

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Detection of OXA-carbapenemase-producing Enterobacteriaceae with chromID CARBA SMART screening plate

Sir,

Carbapenemase-producing Enterobacteriaceae (CPE) have disseminated worldwide and become a global health threat. Various carbapenemase genes have been detected, with significant differences in epidemiology between different geographical locations. In Singapore, OXA-48-like carbapenemases along with NDM and KPC are the three most common carbapenemase genes identified.[1] Phenotypic detection of OXA-48 CPE is fraught with limitations due to relative weaker carbapenemase activity and lower minimum inhibitory concentration (MIC) compared to other carbapenemases. Nonetheless, they are clinically significant. Therefore, the identification and isolation of patients colonised with CPE are integral components of infection control to prevent their spread in both routine and outbreak settings.

The chromID CARBA SMART (bioMérieux, France) is a screening biplate composed of chromID CARBA and chromID OXA-48 agar. The chromID CARBA is a selective agar for CPE with reported sensitivity and specificity of >90% for various non-OXA CPE.[2] Sensitivity as low as 22.8% of the chromID CARBA for detection of OXA CPE has been reported.[3] Girlich *et al.*[3] showed that chromID OXA-48 agar improved the detection of OXA-48-like carbapenemases with a lower limit-of-detection (LOD) of 10 colony forming units (cfu) per plate while the chromID CARBA agar had a LOD of 10^5 cfu per plate for OXA-48 CPE. We report the 2 year experience at a tertiary hospital following implementation of the chromID CARBA SMART.

Retrospective review of laboratory data was performed on CPE screening requests received between 28 August 2015 and 31 August 2017. The laboratory procedures are as follows: rectal swabs were inoculated onto chromID CARBA SMART biplate and incubated at 37°C for 22−24 h. Oxidase-negative Gram-negative organisms were identified with matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF; Bruker Biotyper; Bruker Daltonics, Germany). Disk diffusion testing with imipenem (10 μg), meropenem (10 μg), temocillin (30 μg), and KPC + MBL Confirm ID Kit (Rosco Diagnostics, Denmark) were performed concurrently for all identified Enterobacteriaceae. Isolates with phenotypic features consistent with carbapenemase production include: intermediate or resistant by disk testing to imipenem or meropenem [based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion breakpoints version 5.0 (2015); breakpoints were unchanged in subsequent updates], KPC + MBL Confirm ID Kit (Rosco Diagnostics, Denmark) with combinations consistent with group A serine carbapenemase or group B metallo-β-lactamase (interpreted as per manufacturer instructions), or no zone of inhibition around temocillin disk, and they were subjected to confirmatory PCR for the presence of KPC, NDM, OXA-48, OXA-23, VIM, IMP, and IMI β-lactamase genes as previously described.[4] Duplicate screening tests from the same patient were excluded.

A total of 5025 CPE screening requests were performed within the study period and included in the analysis. A total of 181 requests grew Enterobacteriaceae, of which 29 were negative for CPE based on disk diffusion testing. Polymerase chain reaction (PCR) was performed on the remaining isolates. Seventy-seven patients were found to be carriers of CPE, including 44 NDM, three KPC, 23 OXA, one IMI, and six carrying both NDM and OXA. Of the OXA-CPE carriers, seven patients [9.1% (7/77) of all CPE carriers, and 30.4% (7/23) of all OXA-CPE carriers] had OXA-CPE growing only on the chromID OXA-48 agar. In contrast, samples from three OXA-CPE carriers had growth only on CARBA agar while 13 had growth on both media. All were OXA-48 except for a single OXA-23 CPE.

From the 77 CPE carriers, 93 CPE isolates were detected during this time period (Table 1). NDM was the most commonly isolated carbapenemase gene followed by OXA. Of the OXA-CPE isolates which grew only on chromID OXA-48, seven were *Escherichia coli,* and one was *Citrobacter amalonaticus*.

Use of the chromID CARBA SMART biplate which includes the chromID OXA-48 agar improved detection of OXA-CPE even in a relatively low incidence setting. Our overall CPE-carrier detection rate was 1.5% (77/5025). Of the 77 CPE-colonised patients, 29.9% (23/77) were colonised with OXA-CPE (excluding six patients carrying both NDM and OXA). Seven colonised patients were detected based on the chromID OXA-48 agar alone and the absence of this agar would have resulted in false negative results. However, not all OXA-CPE required chromID OXA-48 for isolation. It is also unclear why three OXA-CPE isolates did not grow on chromID OXA-48 agar. These three isolates included one *Klebsiella pneumoniae,* and two *Enterobacter aerogenes* isolates. The *K. pneumoniae* isolate and one *E. aerogenes* were phenotypically resistant to imipenem and meropenem. The second *E. aerogenes* was phenotypically susceptible to imipenem and meropenem. The first *E. aerogenes* isolate also had an enhanced zone of inhibition around meropenem-boronic acid and meropenem-cloxacillin combinations suggesting the expression of AmpC β-lactamase, although this was not formally tested. No zones of inhibition were seen around temocillin disks for all three isolates. The order of inoculation on the biplate was not specified and its potential impact on outcomes is unknown.

While the overall incidence of CPE in Singapore is increasing, particularly KPC and NDM, the incidence of OXA did not follow the upwards trend of NDM and KPC.[1] This may be influenced by limitations in screening methods and may not reflect true incidence. Overall prevalence may be higher than currently reported rates. Many OXA CPE have relatively lower MICs and may be reported as susceptible based on phenotypic testing alone. Karlowsky *et al.*[5] reported 37.0% of OXA-48-like CPE had imipenem MICs within the susceptible range. However, exposure to carbapenems may rapidly select highly-resistant isolates and the clinical impact of CPE with susceptible MIC is yet to be determined.

Of the OXA-CPE carriers in our patients, 30.4% would not have been detected without the chromID OXA-48 agar. The use of the chromID CARBA SMART can improve detection in settings with low incidence of OXA-CPE. The lack of

Table 1 Summary of isolated carbapenemase-producing Enterobacteriaceae

Carbapenemase gene(s)	Identification	Growth		
		CARBA only	OXA-48 only	CARBA and OXA-48
NDM (52)	*Escherichia coli* (20)	19		1
	Klebsiella pneumoniae (26)	26		
	Enterobacter cloacae complex (4)	4		
	Citrobacter freundii (2)	2		
KPC (3)	*E. coli* (2)	2		
	E. cloacae complex (1)	1		
OXA (27)	*E. coli* (12)		7	5
	K. pneumoniae (12)	1		11
	Enterobacter aerogenes (2)	2		
	Citrobacter amalonaticus (1)		1	
IMI (1)	*E. cloacae* complex (1)	1		
NDM+OXA (10)	*E. coli* (2)	1		1
	K. pneumoniae (6)	3		3
	C. freundii (1)			1
	Kluyvera georgiana (1)			1

adequate screening methods may grossly underestimate prevalence rates. Healthcare providers using agar-based screening methods should evaluate the benefits of using various agars including chromID CARBA SMART for their individual settings.

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MMR protein immunohistochemistry and microsatellite instability in gastric cancers

Sir,

Microsatellite instability-high (MSI-H) is emerging as a new therapeutic target for cancer immunotherapy. In May 2017, the United States Food and Drug Administration (FDA) granted accelerated approval of pembrolizumab for treatment of patients with unresectable or metastatic MSI-H or mismatch repair deficient (dMMR) solid tumours.[1] MSI-H is one major molecular subtype of gastric cancer (GC)[2,3] and is associated with high expression of programmed cell death-ligand 1 (PD-L1).[1,4] Given the relatively high incidence of MSI-H GC in patients with stage III−IV GC,[5] the effective therapeutic use of anti-PD-1/PD-L1 inhibitor in MSI-H GC could contribute to remarkable improvement in survival of patients with GC.

The most accurate method for detecting MSI to date is polymerase chain reaction (PCR), which requires a long test time, is expensive, and is difficult to interpret. So, faster, cheaper, and easily accessible diagnostic algorithms are needed. To investigate how accurately MSI-H cancers can be detected by MMR protein IHC and the distributions of protein losses in dMMR GC, we performed 4 MMR protein IHC and MSI pentaplex test in 580 GC from Asian/Korean patients.

The correlations of MSI, dMMR, and clinicopathological characteristics are summarised in Table 1. The mean age of all 580 patients was 55.6 years (range 24−86), and 385 (66.4%) patients were male. Epstein−Barr encoding region (EBER) *in situ* hybridisation was performed in 569 cases and EBV was positive in 25 cases (4.4%). In Lauren classification, there were 271 (46.7%) diffuse type GCs and 246 (42.4%) intestinal type GCs. There were 11 (1.9%) stage I cancers, 119 (20.5%) stage II cancers, 235 (40.5%) stage III cancers, and 215 (37.1%) stage IV cancers. The mean follow-up duration was 43.6 ± 35.0 months. Of 580 patients, 304 (52.4%) died during the follow up period, and the 5-year survival rate was 36.7%.

In all cases, IHC was performed in representative whole blocks using MLH1 (M1; Ventana, USA) with a BenchMark XT autostainer (Ventana), and MSH2 (G219-1129, 1:500; Cell Marque, USA), PMS2 (MRQ-28, 1:20; Cell Marque), and MSH6 (44/MSH6, 1:500; BD Biosciences, USA) with a Bond-Max autoimmunostainer (Leica Biosystems, Australia). Expression was reported as MMR proficient (pMMR; strong to weak nuclear staining with positive internal controls) or MMR deficient [dMMR; unequivocal loss of nuclear staining or focal (<20%) weak equivocal nuclear staining in the viable tumour cells in the presence of internal positive controls]. For MSI PCR testing, multiplex PCR was performed with five quasi-monomorphic mononucleotide repeat markers in all cases, as previously described.[3,6] Samples with allelic size variation in fewer than two microsatellites were classified as microsatellite-stable (MSS) and allelic size variations in two or more microsatellite markers were considered MSI-H. For cases with discrepant results between IHC and PCR tests, both experiments were repeated three times and the consistent results were used for final analyses.

For statistical analyses, we used age, sex, histological type by Lauren classification, World Health Organization (WHO) tumour classification, TNM stage (AJCC 8th edition), and overall survival (OS) of patients as clinical variables with the SPSS 24.0 statistical software program (IBM, USA). MSI/dMMR and clinicopathological variables were compared using Pearson's chi square test and were further analysed with linear-by-linear association. The Kaplan−Meier method was used to estimate OS. p values less than 0.05 were considered statistically significant.

MSI-H was found in 60 cases (10.3%) and dMMR was observed in 61 cases (10.5%) out of 580 GC (Fig. 1). All EBV-positive GC was pMMR and MSS. Both dMMR and MSI-H were significantly correlated with old age ($p<0.001$), low AJCC stage ($p<0.003$), intestinal-type by Lauren classification ($p<0.005$), and longer OS ($p<0.001$) (Table 1, Fig. 2). In multivariate analyses, dMMR and MSI-H were independent favourable prognostic factors ($p = 0.001$) in addition to AJCC stage (Supplementary Table 1, Appendix A).

In MMR IHC, 61 dMMR cases consisted of 52 MLH1/PMS2 losses (85.2%), four MSH2/MSH6 losses (6.6%), and