Antimicrobial Resistance Investigation on *Staphylococcus* Strains in a Local Hospital in Guangzhou, China, 2001–2010

Yang Deng,¹ Junyan Liu,¹ Brian M. Peters,² Lei Chen,³ Jian Miao,¹ Bing Li,¹ Lin Li,¹
Dingqiang Chen,⁴ Guangchao Yu,⁵ Zhenbo Xu,^{1,6} and Mark E. Shirtliff^{6,7}

A retrospective study was conducted on 1,739 *Staphylococcus* isolates from the First Affiliated Hospital of Jinan University (FAHJU) in Guangzhou during 2001–2010. With the exception of teicoplanin and vancomycin, antimicrobial resistance was commonly observed among the isolates examined, with high resistance rates for β -lactamases (94.0% and 73.7% for penicillin and oxacillin) and resistance percentages for cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, trimethoprim–sulfamethoxazole, and tetracycline ranging from 83.9% to 19.4%. Two hundred sixty-three of the 1,739 isolates were subjected to SCCmec typing and 42 to MLST, *spaA*, and *coa* typing. ST239-MRSA-III was prevalently identified along with one distinct *coa* type HIJKL and 2 *spaA* types (WGKAOMQ-t037 and WGKAQQ-t030). Class 1 integrons were commonly detected (31.6%), although none of the integron-positive MRSA strains had been isolated since 2009. The widespread detection of integron-based antimicrobial resistance determinants may further contribute to the emergence of superbugs.

SINCE IT WAS FIRST DISCOVERED in 1961, methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major global health concern and has been responsible for a large variety of bacterial infectious diseases.^{3,4,9,10} Considered to be the primary agent in the spread and dissemination of multidrug resistance among bacteria, integrons have been frequently detected among clinical gram-negative microorganisms.² Nevertheless, in the past decade, integrons have also been commonly detected in gram-positive microorganisms and found to contribute to their multidrug resistance, including erythromycin, gentamicin, tetracycline, and trimethoprim–sulfamethoxazole.^{7,15,16} Despite their frequent identification among MRSA strains in Guangzhou, Southern China, during 2001–2006,^{12,13} the contribution of integrons to antimicrobial resistance among recently isolated *Staphylococcus* strains has not been evaluated. Thus, in this study, a retrospective investigation of *Staphylococcus* strains from the First Affiliated Hospital of Jinan University (FAHJU) isolated over a 10-year period from 2001 to 2010 was carried out. In this study, antimicrobial resistance pro-

files, MRSA typing (SCCmec, multilocus sequence typing [MLST], *spaA*, and *coa*) for selected isolates, and integron screening were conducted on the more recently isolated MRSA strains.

From 2001 to 2010, a total of 1,739 clinical *Staphylococcus* isolates were obtained from FAHJU in Guangzhou, Southern China, with 1,131 *S. aureus* and 608 coagulase-negative staphylococci (CNS) strains. Bacterial identification was performed by the API Staph strip test and Vitek 2 automated system. Antimicrobial susceptibility testing was performed by the standard disk diffusion method, and minimum inhibitory concentrations were determined for 12 antibiotics according to Clinical Laboratory and Standards Institute (CLSI) methods¹ (Table 1). In this study, resistance rates against β -lactamases along with other antimicrobials were found to be high, with the exception of teicoplanin and vancomycin (Table 1). It is worth noting that when resistance rates were compared between *S. aureus* and CNS strains, significant differences were observed in relation to clindamycin, cefoxitin, trimethoprim–sulfamethoxazole, and

¹College of Light Industry and Food Sciences, South China University of Technology, Guangzhou, China.

²Department of Oral Biology, School of Dentistry, Louisiana State University Health Sciences Center, New Orleans, Louisiana.

³Institute of Agro-products Processing, Anhui Academy of Agricultural Sciences, Hefei, China.

⁴Department of Laboratory Medicine, First Affiliated Hospital of Guangzhou Medical College, Guangzhou, China.

⁵Clinical Laboratory Center, The First Affiliated Hospital of Jinan University, Guangzhou, China.

⁶Department of Microbial Pathogenesis, School of Dentistry, University of Maryland, Baltimore, Maryland.

⁷Department of Microbiology and Immunology, School of Medicine, University of Maryland, Baltimore, Maryland.

TABLE 1. ANTIMICROBIAL RESISTANCE OF *STAPHYLOCOCCUS* ISOLATED IN SOUTHERN CHINA DURING 2001–2010

Bacterial	Antimicrobial resistance rate											
	CEF	CHL	CIP	CLI	ERY	GEN	PEN	OXA	SXT	TEI	TET	VAN
<i>Staphylococcus aureus</i>	4.2%	24.3%	73.1%	70.3%	84.6%	60.3%	96.3%	68.9%	35.0%	4.8%	61.3%	0.8%
CNS	47.7%	34.6%	67.1%	65.3%	82.5%	51.9%	89.7%	82.7%	50.6%	2.0%	55.0%	0.7%
Total	19.4%	27.9%	71.0%	68.5%	83.9%	57.4%	94.0%	73.7%	40.5%	3.8%	59.1%	0.7%

Twelve antibiotics were used in establishing the level of antimicrobial resistance among the *Staphylococcus aureus* (1,131 strains) and CNS isolates (608 strains). The antibiotics examined included CEF, cefoxitin (30 µg); CHL, chloramphenicol (30 µg); CIP, ciprofloxacin (5 µg); CLI, clindamycin (2 µg); ERY, erythromycin (15 µg); GEN, gentamicin (10 µg); OXA, oxacillin (1 µg); PEN, penicillin (10 µg); SXT, trimethoprim–sulfamethoxazole (1.25/23.75 µg); TEI, teicoplanin (30 µg); TET, tetracycline (30 µg); VAN, vancomycin (30 µg).

CNS, coagulase-negative staphylococci.

chloramphenicol. SCCmec typing was then performed on 263 randomly selected MRSA strains (data obtained within the period from 2001 to 2006 had been partially reported in a previous study¹³) and further validated by multiplex PCR assays.^{5,6,8} Type III SCCmec was most frequently observed with an identification rate of 94.7% (249/263), with Type II detected in four isolates (one individual isolate in 2001, 2002, 2005, and 2008, respectively) and 10 untypeable MRSA strains were recorded. MLST along with *spaA* and *coa* typing was also conducted on 42 representative MRSA isolates (selected by sampling year, SCCmec types, and carriage of integrons with cassettes),^{11,13} with all the detected strains identified to be the ST239-MRSA-III group (clonal complex 239, CC239), *coa* type HIJLK, as well as two *spaA* types (WGKAOMQ-t037 for 35 strains and WGKAQQ-t030 for 7 strains, respectively). Integron characterization was performed as described previously,¹³ and class 1 integrons were commonly found in MRSA strains (31.6%, 83/263), with decreasing identification rates observed over time: 65.0% (13/20) in 2001, 55.0% (11/20) in 2002, 46.7% (7/15) in 2003, 44.0% (15/34) in 2004, 36.0% (18/50) in 2005, 30.0% (12/40) in 2006, 22.2% (4/18) in 2007, 13.6% (3/22) in 2008, and 0% (0/44) in 2009 and 2010. An extensive integrons study was further conducted on 26 MRSA strains from FAHJU during 2011–2012 and 260 MRSA strains from the First Affiliated Hospital of Guangzhou Medical College (FAHGMC, another tertiary hospital setting in Guangzhou) during 2006–2010, with no integrons identified within the MRSA isolates analyzed. However, from another retrospective integron investigation on a total of 583 clinical microorganisms from FAHJU during 1998–2006, class 1 integrons were found in 73.6% (243/330) and 49.0% (124/253) of the gram-negative and gram-positive bacteria, totaling a prevalence rate of 63.0% (367/583). Individual genera and species integron identification rates ranged from 83% to 92%: *Escherichia coli* (89.3%, 109/122), *Klebsiella pneumoniae* (87.5%, 28/32), *Acinetobacter* spp. (91.3%, 21/23), *Enterobacter cloacae* (86.7%, 13/15), *Enterococcus* spp. (86.7%, 13/15), *Streptococcus* spp. (83.3%, 5/6), and other gram-negative organisms (90%, 18/20).^{11,14} Results were notably lower for other common nosocomial pathogens, including *Pseudomonas aeruginosa* (45.8%, 54/118), *S. aureus* (42.5%, 76/179), and CNS (56.6%, 30/53) isolates.^{11,14} In addition, class 2 integrons were occasionally observed occupying 5.7% (33/583) of all isolates, including 23 *P. aeruginosa*, 6 *E. coli*, 2

Enterococcus faecalis, 1 *Proteus vulgaris*, and 1 *Proteus mirabilis* strains, with identical *dfrA1-sat1-aadA1* cassette arrays obtained for all strains. The most frequently detected resistance genes were the *aadA* (88.3%, 324/367) and the *dfrA* (74.9%, 275/367) family. The identification rates of cassette arrays were 54.5% (200/367) for *dfrA12-orfF-aadA2*, 18.8% (69/367) for *dfrA17-aadA5*, and 16.1% (59/367) for *aadA2*, respectively. Despite none of the tested strains being positive for canonical class 3 integrons, one *S. aureus* strain isolated from sputum in 2003 was found to be positive for the *intI3* variable region but lacked both flanks typical of the class 3 integron structure. This surprising result may provide insight into the evolution of integron-mediated resistance mechanisms among various bacterial strains, although more thorough studies are required.

Importantly, the identification of integrons has been significantly limited by study setting and time span, as none of the integron-positive MRSA strains were found recently (since 2009) from either FAHJU or other medical settings such as FAHGMC. Similarity of integron occurrence between gram-negative and gram-positive microorganisms, high homology of such acquired array cassettes, as well as laboratory observation of excision and integration events mediated by class 1 integrons in *Staphylococcus*¹⁴ have strongly suggested the horizontal transfer of resistance gene cassettes through class 1 integrons among the *Staphylococcus* population. In addition, such site-specific recombination, including both *intI1*-mediated excision of 4 different gene cassettes (*dfrA1-aadA1*, *aadA1*, *cmlA1*, and *aadB1*) by different efficiencies and further specific integration into the G/TTRRRY consensus of *attI*, has also been verified for the recipient *S. aureus* strain RN4220 (unpublished data). Southern hybridization analysis on 58 randomly selected *Staphylococcus* strains (30 MRCNS¹⁵ and 28 MRSA strains) has demonstrated the location of class 1 integrons (including *intI1* and associated gene cassettes) as located on chromosomes, and not plasmids. Such discovery requires further investigation on the horizontal gene transfer between gram-positive and gram-negative microorganisms as well as the mechanisms of integron mobility in *Staphylococcus* isolates. Despite the recent absence of integron identification in MRSA, the carriage of such elements within *Staphylococcus* (MRSA during 2001–2008 and CNS during 2001–2004) strains confer resistance to multiple antimicrobials. Thus, commonly detected integron-based antimicrobial resistance

mechanisms may further contribute to the dissemination of new waves of superbugs.

Acknowledgments

The authors sincerely thank Dr. Jin Chu from the University of Leeds for her excellent support for this study. This work was supported by the National 973-Plan of China (2012CB720800), the National Natural Science Foundation of China (31201362 and 31101278), the National Science and Technology Support Program (2012BAD37B01), the National Outstanding Doctoral Dissertation Funding (Dr. Zhenbo Xu), the Guangdong Outstanding Doctoral Dissertation Funding (K3140030), the China Postdoctoral Science Foundation funded project (2014T70810 and 2013 M542182), and the Fundamental Research Funds for the Central Universities (2012ZM0060 and 2013ZB0021).

Disclosure Statement

All authors report no conflicts of interest relevant to this article.

References

1. **Clinical and Laboratory Standards Institute.** 2005. Performance standards for antimicrobial susceptibility testing: 15th informational supplement CLSI/NCCLS document M100-S15. Clinical and Laboratory Standards Institute Wayne, PA.
2. **Fluit, A.C., and F.J. Schmitz.** 1999. Class 1 Integrins, gene cassettes, mobility, and epidemiology. *Eur. J. Clin. Microbiol. Infect. Dis.* **18**:761–770.
3. **Foster, T.J.** 2004. The *Staphylococcus aureus* “superbug.” *J. Clin. Invest.* **115**:1693–1696.
4. **Gomez, J., E. Garcia-Vazquez, R. Banos, M. Canteras, J. Ruiz, V. Baños, J.A. Herrero, and M. Valdés.** 2007. Predictors of mortality in patients with methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia: the role of empiric antibiotic therapy. *Eur. J. Clin. Microbiol. Infect. Dis.* **26**:239–245.
5. **Hisata, K., K. Kuwahara-Arai, M. Yamanoto, T. Ito, Y. Nakatomi, and L. Cui.** 2005. Dissemination of methicillin-resistant *Staphylococci* among healthy Japanese children. *J. Clin. Microbiol.* **43**:3364–3372.
6. **Ito, T., Y. Katayama, K. Asada, N. Mori, K. Tsutsumimoto, and C. Tiensasitorn.** 2001. Structural comparison of three types of staphylococcal cassette chromosome *mec* integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **45**:1323–1336.
7. **Nandi, S., J.J. Maurer, and C. Hofacre.** 2004. Gram-positive bacteria are a major reservoir of class 1 antibiotic resistance integrons in poultry litter. *Proc. Natl. Acad. Sci. U. S. A.* **101**:7118–7122.
8. **Oliveira, D.C., and H. Lencastre.** 2002. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **46**:2155–2161.
9. **Rojo, A., A. Aguinaga, S. Monecke, J.R. Yuste, G. Gastaminza, and A. España.** 2014. *Staphylococcus aureus* genomic pattern and atopic dermatitis: may factors other than superantigens be involved? *Eur. J. Clin. Microbiol. Infect. Dis.* **33**:651–658.
10. **Schito, G.C.** 2006. The importance of the development of antibiotic resistance in *Staphylococcus aureus*. *Clin. Microbiol. Infect.* **12** (Suppl. 1):3–8.
11. **Xu, Z.** 2011. Investigation on molecular mechanism of toxicity and antibiotic resistance in *Staphylococcus* biofilm behavior in sugar biosafety [Ph.D. dissertation]. South China University of Technology, Guangzhou, China.
12. **Xu, Z., L. Li, L. Shi, and M. Shirliff.** 2011. Class 1 integron in staphylococci. *Mol. Biol. Rep.* **38**:5261–5279.
13. **Xu, Z., L. Li, M. Shirliff, B.M. Peters, B. Li, Y. Peng, M.J. Alam, S. Yamasaki, and L. Shi.** 2011. Resistance class 1 integron in clinical methicillin-resistant *Staphylococcus aureus* strains in Southern China, 2001–2006. *Clin. Microbiol. Infect.* **17**:714–717.
14. **Xu, Z., L. Li, G. Yu, L. Shi, B. Li, and J. Su.** 2012. Retrospective summary report of integron study in a local hospital in southern China. Abstract of the 22nd European Congress of Clinical Microbiology and Infectious Diseases, London, UK.
15. **Xu, Z., L. Shi, M.J. Alam, L. Li, and S. Yamasaki.** 2008. Integron-bearing methicillin-resistant coagulase-negative staphylococci in South China, 2001–2004. *FEMS Microbiol. Lett.* **278**:223–230.
16. **Xu, Z., L. Shi, C. Zhang, L. Zhang, X. Li, Y. Cao, L. Li, and S. Yamasaki.** 2007. Nosocomial infection caused by class 1 integron-carrying *Staphylococcus aureus* in a hospital in South China. *Clin. Microbiol. Infect.* **13**:980–984.

Address correspondence to:

Zhenbo Xu, PhD

College of Light Industry and Food Sciences

South China University of Technology

381 Wushan Road

Guangzhou 510640

China

E-mail: zhenbo.xu@hotmail.com