Report for ESCMID Training in a Foreign Institution

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Project title: Application of molecular typing methods for studying methicillin-resistant *Staphylococcus aureus* infections

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Introduction:

The ability to quickly and reliably differentiate among related bacterial isolates is essential for epidemiological surveillance, and is an endeavor as old as the discipline of bacteriology itself. Long-standing conventional typing methods, such as bacteriophage typing of *Staphylococcus aureus* and *Listeria monocytogenes* [1,2], serotyping of *Salmonella* spp. and *Escherichia coli* [3,4], or biochemical typing of Enterobacteriaceae [5], have historically been important contributors to our understanding of the natural history and epidemiology of infections caused by strains of these clinically relevant bacterial species. Similarly, antibiotic susceptibility testing has for many years been and, still is, in the field of clinical microbiology, a first-line method to identify possible cases of bacterial cross-transmission in healthcare institutions. These phenotypic methods for bacterial typing have a clear purpose in the confirmation and elucidation of local and national healthcare-associated outbreaks [6].

The rate of genetic exchange within many bacterial species means that a given phenotype may not always reflect evolutionary history. For example, two isolates that are identical according to phage typing might in fact be quite unrelated, and conversely, two isolates that show quite different phenotypes for a single marker might in fact be closely related. For these reasons, phenotyping has been largely replaced by genotypic or 'molecular' typing over the past two decades [9]. In principle, at least, asexual (clonal) reproduction by binary fission implies that genotypic markers should reflect evolutionary history and would therefore be useful in delineating a natural taxonomy.

Experimental objectives:

An ESCMID 2008 Travel grant for training in foreign institution assisted me in this training on molecular typing in the Department of Microbiology, School of Medicine, University of Patras in Greece. Training included learning the techniques of PCR and PFGE and using the methods to test representative MRSA strains previously characterized in our laboratory in Nigeria as well as from the Host Laboratory (strains received between April-June, 2008).

Methods:

The MRSA strains were selected according to their phenotypic characteristics (antibiotic resistant profiles), susceptibility to oxacillin by E-test (AB Biodisk), catalase, coagulase, and detection of β-lactamase. This was verified by a latex agglutination test (bioMerieux) for PBP2a production combined with PCR for *mecA* gene carriage.

DNA was extracted by suspending portions of four to five colonies in 1ml dH_2O equivalent to 2MF, centrifuged for 10mins at 13000rpm. The supernatant was rejected and the sediment resolved with 100ml lysis buffer (50mM Tris-HCl pH=7.5, 1% Triton X-100, 1mM EDTA pH=8.0) + 0.2 µl proteinase K (1µl/ml from stock 20mg/ml). Initial incubation was done at 56°C for 1 hour followed by 95°C for 10mins. The DNA extracts were stored at -20°C prior to use. PCR for the presence of the gene *lin* was used to test for the quality of the DNA extracts.

Classification of MRSA strains was performed by PCRs according to *agr* groups [10]. The presence of toxin genes (*tst*, PVL, *egc*) was investigated by PCRs using specific primers and programmes [11]. Chromosomal DNA extraction of MRSA was performed into agarose disks and clonal types was defined by PFGE of *Sma*I digests [12,13,14].

Results:

A total of 78 representative MRSA strains (comprising 74 from Host Laboratory and 4 from Nigeria) were used in this study. Four PFGE types, three *agr* allele types were detected among the 74 MRSA strains from the host laboratory while two PFGE types and one *agr* type from the Nigeria strains (Table 1). Among the strains from Greece, there is strong correlation between PFGE type C and *agr* 3 with resistance to kanamycin and fusidic acid. No toxin genes were detected from the Nigerian strains however the PFGE clonal types were different from that of Greece.

As planned, the training yielded a good knowledge transfer as I can now plan, perform, interpret molecular typing methods as well apply the methods for bacterial characterization.

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Table : Results of genotypic and phenotypic characterization of the 78 MRSA strains.

HOST INSTITUTION

PFGE type	*agr*	Antibiotic resistance	Toxin genes	No. of strains
C	3	K, FA	PVL*, sem, seg*	46
		multiresistance		18
G	3	multiresistance	PVL*, sem, seg*	4
	2			1
B	1	multiresistance	*sem, seg*	4
A	3	S	*seg*	1
TOTAL				**74**

NIGERIA STRAINS

PFGE type	*agr*	Antibiotic resistance	Toxin genes	No. of strains
H	1	K, ER, SXT	-	2
L	1	multiresistance	-	2
TOTAL				**4**

K- kanamycin, FA- fusidic acid, S- streptomycin, ER-erythromycin, SXT-co-trimoxazole; multiresistance- resistance to more than three antibiotic groups

Figure 1: PCR agarose gel electrophoresis

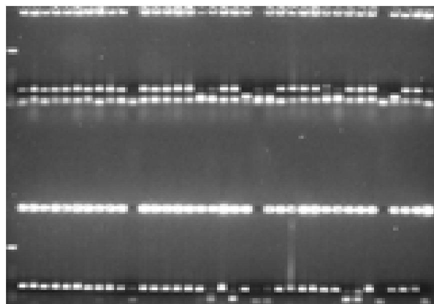

Figure 2: PFGE