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Abstract (poster session)

MRSA harbouring mecA-LGA251, a new highly divergent mecA variant: performance of the methods used in routine labs to screen, detect and confirm methicillin resistance

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Background: A new mecA variant, named mecA-LGA251, showing less than 70% homology with the classical mecA gene, has recently been described in methicillin-resistant Staphylococcus aureus (MRSA) isolates from human and animal. We investigated the performance of different phenotypic and genotypic methods routinely used in microbiological labs to screen, detect, and confirm the presence of isolates harboring such a methicillin-resistance (MR) mechanism. Methods: A large collection including 111 mecA-LGA251-positive isolates were collected in UK, Scotland, Denmark and France. Four chromogenic MRSA selective media (MRSA Select (bR), ChromID MRSA (bM), BBL CHROMagar MRSA II (BD), Brilliance MRSA 2 (Oxoid)) were tested for screening. Antimicrobial susceptibility tests (AST) included MIC for FOX and OXA using BMD, AST-P581 (Vitek), PMIC/ID-60 (Phoenix), Pos MIC Panel Type 31 (Microscan). Immunological detection of additionnal PBP were performed using Clearview Exact PBP2a (Alere) and PBP2a agglutination (Oxoid). Finally, molecular tests, including ”homebrew” mecA PCR, BD GeneOhm StaphSR assay (BD), Xpert MRSA/MSSA SSTI and nasal (Cepheid), NucliSENS EasyQ MRSA (bM) and DNA microarray StaphyType (Alere), were performed. Results: The 111 isolates belonged to CC130 (n= 92, agr 3, 16 spa-types), CC1943 (n=14, agr 4, 4 spa-types) and CC425 (n=5, agr 2, 4 spa-types). All were MR but susceptible to all the other antibiotics tested. Data highlighted a highly variable sensitivity for the various selective media and AST tested (see Table 1). Clearview Exact PBP2a test, performed after cefoxitin induction (disc), were the only method allowing the confirmation of expression of additionnal PBP in all isolates. None of the homebrew mecA PCR or commercial molecular kits currently available was able to identify these isolates. Using DNA microarrays (n=37), assignment to the specific clones known to be positive for mecA-LGA251 gene were achieved and data revealed the seldom presence of some toxins and virulence genes : tst (n=7), egc (n=9), edinB (n=8), sec (n=3), sel (n=3). Conclusion: The data presented demonstrates that i) the ability of commercial methods used to screen, identify or confirm mecA-LGA251-positive isolates is highly variable, ii) such isolates may be missed depending on the used algorithms. The only ways to definitively confirm the meticillin-resistance in such isolates are the use of specific mecA-LGA251 PCR or Clearview Exact PBP2a after cefoxitin induction.

CC	agr	n	Number of different spa	Antimicrobial susceptibility test						Immuno chromatography		Screening selective media (number of MRSA detected after 24h)			
				Vitek		Phoenix		Microscan		Clearview without induction	Clearview with induction	MRSA Select (bR)	Brilliance MRSA (Oxoid)	ChromAgar MRSA (BD)	ChromID MRSA (bM)
				MSSA	MRSA	MSSA	MRSA	MSSA	MRSA						
130	3	**92**	16	2	90	22	70	3	89	10	92	62	89	74	91
1943	4	**14**	4	1	13	1	13	1	13	0	14	6	14	9	14
425	2	**5**	5	0	5	3	2	0	5	0	5	2	5	5	5
Total correctly identied (%)				**97.3**		**76.6**		**96.4**		**9.1**	**100**	**63.6**	**97.3**	**80**	**99.1**

Table 1 : Data obtained for a collection of 111 European MRSA isolates harboring mecA-LGA251 gene.