Development of optimal algorithm for determination of *Escherichia coli* and *Klebsiella pneumoniae* with phenotypic $ESBL_A$ and $ESBL_M$ based method

Ülle Parm (MD; PhD)[1,2], Sirje Laks[3], Anna-Liisa Tamm (PhD)[2], Svetlana Rudenko (MD)[3]

Department of Microbiology , University of Tartu - 1; Tartu Health Care College - 2, The North Estonia Medical Centre -3

Objective

The spread of β-lactamase-producing (ESBL) *Enterobacteriaceae* is the contemporary worldwide problem. For better results in infection prevention and therapy antimicrobial-resistance surveillance is needed.

Aim

to compare results of different β-lactamases phenotypic screening and confirmatory tests (screening disks, combined disks and gradient strips) used in clinical microbiology laboratories.

Material and Methods

•Altogether, 171 strains of bacteria (78 *Escherichia coli*; 93 *Klebsiella pneumoniae*) with decreased sensitivity to third generation cephalosporines (cephotaxime; ceftasidime; cefepime; cefoxitine; cefpodoxime) were enrolled the study. Clavulanic acid and cloxacillin gradient stripes (Liofilchem) and combined disks from companies Rosco and MAST were used to determine the mechanisms of resistance.

Table. Combinations of antibiotics for phenotypic determination of resistance

	$ESBL_A$	$ESBL_M$	$ESBL_{AM}$
Rosco	CTX30; CAZ30; CTX+C; CAZ+C	CTX30; CAZ30; CTX+CX; CAZ+CX	CTX30; CAZ30; CTX+CX; CAZ+CX; CTX+C;CAZ+C
Mast	B - CPD + ESBLA inhibitor	C - CPD + ESBLM inhibitor	D - CPD + ESBLAM inhibitor
Liofilchem	CAZ/CAL; CTX/CTL; FEP/FEL	CTT/CXT	CAZ/CAL; CTX/CTL; FEP/FEL; CTT/CXT

CTX30 - cephotaxime 30 µg; CTX+C - cephotaxime 30 µg + clavulanic acid; CTX+CX - cephotaxime 30 µg + cloxacillin; CAZ 30 - ceftazidime 30 µg; CAZ+C - ceftazidime 30 µg + clavulanic acid; CAZ+CX - ceftazidime 30 µg + cloxacillin; CTT/CXT – cefotetan / cefotetan+cloxacillin; FEP/FEL – cefepime / cefepime+clavulanic acid; CTX/CTL – cefotaxime / cefotaxime+clavulanic acid; CAZ/CAL – ceftazidime / ceftazidime+ clavulanic acid; CPD - cefpodoxime 10µg.

Results

The most frequent β-lactamase was $ESBL_A$. Outcome of all used three tests showed the matching $ESBL_A$ in 70 of 152 any positive cases got from different tests that were 41% of all strains.

Gradient stripes showed statistically more $ESBL_A$ but less $ESBL_{AM}$ strains in comparing with Rosco and MAST tests.

The MAST tests showed more $ESBL_M$ strains in comparing with gradient stripes.

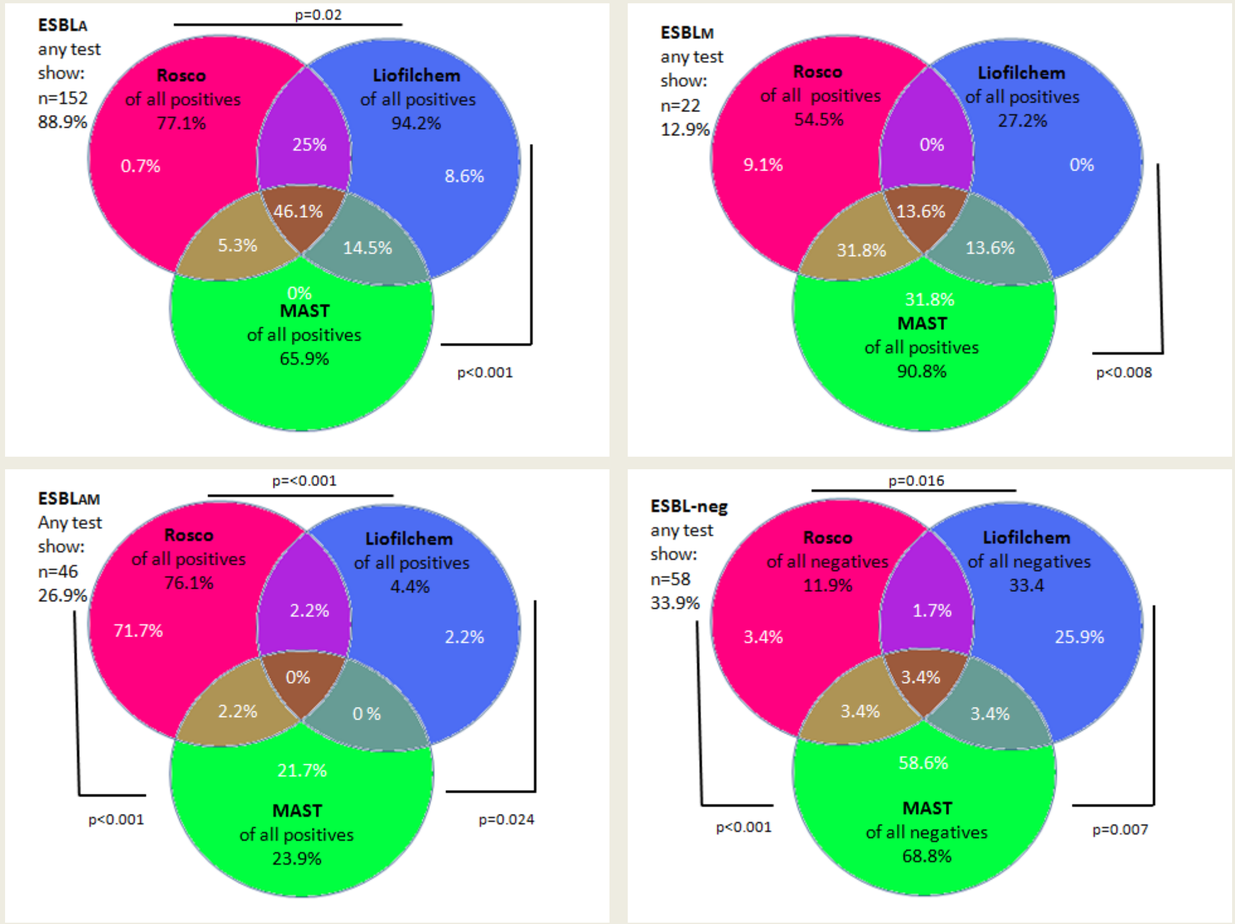

Figure. Differences in determining $ESBL_A$, $ESBL_M$ and $ESBL_{AM}$ in comparing with MAST and Rosco tests, and gradient strips. In calculating p value all studuy group (n=171) were considered.

Only three strains were detected as $ESBL_M$ with listed all three tests and for $ESBL_{AM}$ strains there were no matching results. With all three tests two strains gave similarly negative results (1%).

Conclusions

Usage of different phenotypic tests gives different results. Due to the price and work load, it would be beneficial to use the combined discs of MAST. However, until there is no molecular confirmation, we can not give recommendations.