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Phenotypic and genotypic profile of hospital bacteria isolated from four Moroccan hospitals between 2011 and July 2013

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ABSTRACT: During 2011-2013, 189 bacteria from four Moroccans regional hospitals were referred to The National Institute of Hygiene (NIH) to achieve phenotyping and genotyping.
Galleries Api20E, Api20NE and Api20Sthap (bioMérieux), were used for Biochemical identification and disks distribution method was used for antibiotic susceptibility testing.
Genotyping was carried out by molecular biology by searching genes of resistance (CTX, SHV and TEM) for enterobacteria, and gene mec A for the *staphylocoque*. among Enterobacteriaceae *Escherichia coli* was prevalent, followed by *pseudomonas aerugenosa, sthaphylococoque, klebsiella* pneumonaie and *acenitobacter baumanii*. The second common phenotype is expanded spectrum lactamase"ESBL».
CTX gene was the most predominant for enterobacteria ESBL with an expression about 43.6%.
The gene responsible for resistance to Methicillin in our case was mec A which detected in the six strains tested Methicillin-resistant S. aureus (MRSA).

KEYWORDS: enterobacteria, phenotyping, genotyping, genes, resistance.

1 INTRODUCTION

Hospital infections represent a worldwide worrisome problem. In addition, antimicrobial resistance increased morbidity, mortality, and cost of health care.

Thus, the establishment of monitoring bacterial resistance system has become one of the most important supports recommended in the guidelines for the prevention against antimicrobial resistance in hospitals.

On the other hand, information about resistance trends, including emerging antibiotic resistance, is essential for clinical practice. Such information could be provided by the routinely susceptibility testing of bacterial isolates and the antibiotic resistance surveillance.

The infections are more challenging when caused by antimicrobial-resistant bacteria [1],[2],[3],[4]. Among these resistant bacteria, extended-spectrum cephalosporin-resistant Enterobacteriaceae, carbapenem-resistant, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, and ciprofloxacin-resistant Enterobacteriaceae and non-fermentative Gram-negative bacilli (NFGNB) are of great concern. In fact, antimicrobial therapy for infections due to these resistant pathogens remains a clinical dilemma within hospitalized patients [5] ,[6] ,[7] ,[8],[9],[10],[11].

Among the most relevant emerging resistances in hospitals from the United States and Europe are methicillin-resistance, and more recently, glycopeptides resistance staphylococci, gentamicin and glycopeptides resistance enterococci, as well as

the resistance to fluoroquinolones,extended-spectrum cephalosporins, and carbapenem displayed by gram-negative bacilli [12].

The antibiotic susceptibility profiles of bacterial isolates are unknown in much of the developing world. The objective of this study is the determination of the phenotypic and genotypic profile of nosocomial bacteria isolated from four Moroccan hospitals.

2 MATÉRIEL ET MÉTHODES

2.1 SAMPLING

Prevalence study was conducted During 2011-2013 by four Moroccan centers. Antimicrobial susceptibility data of clinically relevant isolates from patients (48hr hospitalization) were collected in a computerized system . Sex, age, hospital ward, and the type of specimen were recorded for every patient. Underlying clinical condition, the source and type of the infection, as well as previous antimicrobial therapy were also recorded when available.

The Nosocomial infections unit has for mission to ensure the microbiological investigation of nosocomial infections and to determine the profile of resistance, Identification and genotyping for all strains were carried out from four provinces at level 4 hospital sites.

During 2011-july 2013 our laboratory received 189strains for confirmation and identification sensitivity.

The Bacteria were identified according to standard procedures. Susceptibility testing was carried out by disk diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) guidelines13.

2.2 DNA EXTRACTION

DNA was extracted by heat shock at 100°C dry bath for 10 minutes and then cooled immediately in ice. After 10 minutes of centrifugation at 12,000 rounds / minute, the supernatant was stored at-20 ° C until use Thabaut et al [14]

2.3 POLYMERASE CHAIN REACTION (PCR)

Amplification of genes encoding CTX - M group 1, TEM, SHV enzyme was made using "primers" specific (table I).

The amplification was made into a thermocycler, the mix for PCR reactions(CTX - M group 1, TEM, SHV) being composed of a unit of Taq polymerase, 0.4 mM of each primer, 100 mM of each deoxynucleoside triphosphate, 0.5 mM MgCl2, 10 mM Tris - HCl pH 8.3 and 50 mM KCl. One microliter of the DNA test was added to a final volume of 50 µl.

The reaction was made in a thermal cycler (PROGEN), on terms of amplifications.

2.4 REVELATION OF THE PCR PRODUCT BY AGAROSE GEL ELECTROPHORESIS

An agarose gel with concentration of 1% was used for electrophoresis, TBE 1 X buffer was used for gel preparation (Tris Borate EDTA), migration was performed by a current generator (Consort), voltage of 90 Volts for 30-40 minutes was used. The gel was visualized using a transilluminator to UV).

Table 1. amorces for CTX-M, SHV TEM

les gènes		Primer
CTX-M	CTX-MF	50 - ATGTGCAGYACCAGTAARGT – 30
	CTX-MR	50 - ACCGCRATRTCRTTGGTKGT – 30
TEM	TEML	50 - ATGAGTATTCAACATTT – 30
	TEMR	50 - TTACCAATGCTTAATCA – 30
SHV	OS5	50 - TTATCTCCCTGTTAGCCACC – 30
	OS6R	50 - GATTTGCTGATTTCGCTCGG – 30
Mec A		• 5’-GCGATCAATGTTACCGTAG-3’ ;
		• 5’-AGAAATGACTGAACGTCCG-3’

Table 2. programme of reaction

Gene	initial step	Denaturation, hybridization, stretching	the number of cycles	final step
CTX-M	94°C 5 min	94°C 1min/60°C 1min/ 72°C 1min	30	72°C 7min
SHV	94°C 5 min	94°C 1min/60°C 1min/ 72°C 1min	25	72°C 7min
TEM	94°C 5 min	94°C 1min/50°C 1min/ 72°C 1min	25	72°C 7min
Mec A	95°C 9 min	94°C 30s/55C 30s/ 72°C 90s	30	72°C 7min

3 RESULTS AND DISCUSSION

3.1 THE BACTERIA IDENTIFIED

All bacteria which were sent from four provinces indicated that Enterobacteriaceae (42.3%) was prevalent, ESBL Enterobacteriaceae representing 43.6% (E coli 45%). (figure 1 and 2)

Followed by pseudomonas aeurogenosa with 18% ,which Resisters occupy 75%.

In third position was sthaphylococoque had 14.8% (24% had a Methicillin-resistant).

The incidence of MRSA strains is high particularly in Japan, being 60% nationwide, and as high as 90% in individual hospitals (15).

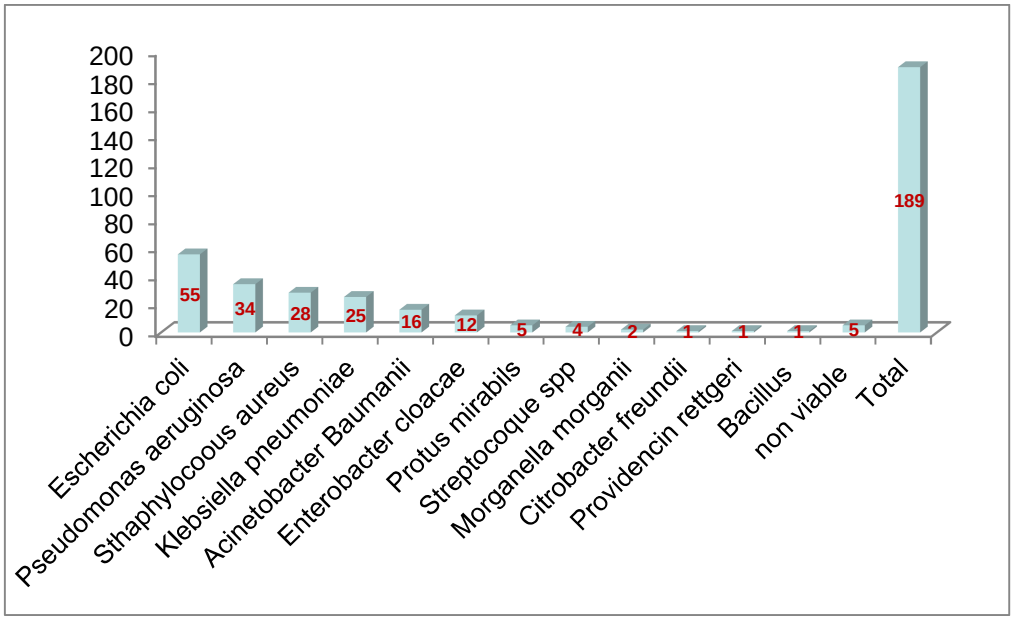

Figure1 :The bacteria identified

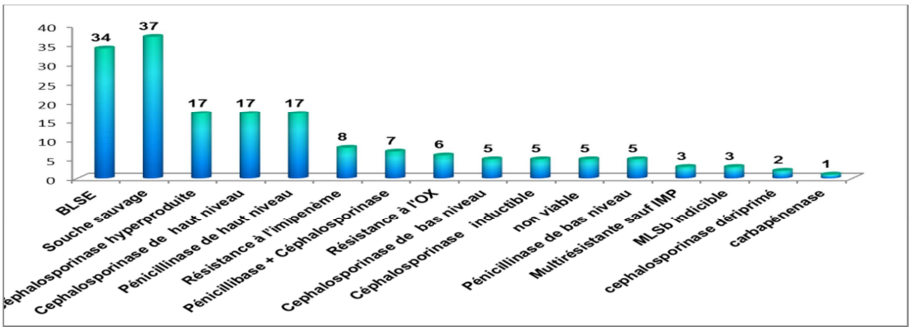

Figure 2 : Strains of fourths sites identified according to their phenotype

In European countries the rate of MRSA was found to be 46% in Denmark, (16) 22% in Poland, (17) 46-67.5% in Greek hospitals (18).,26-33% in Italy,(19). and 3% in the North Middlesex Hospital, UK.(15) In Turkey, the prevalence of MRSA strains has ranged from 25-80% (20)

3.2 GENES FOR ESBL

The responsible genes for ESBL production were CTX-M, SHV and TEM. sometimes two genes or more were found.

CTX was the most prevalent gene, it represented 51.5% of ESBL Enterobacteriaceae,

And in combination with TEM and SHV it was 31.5% of these bacteria.

SHV was represented only 8.6 % and in combination with CTX it was 14.3%.(table 2)

Table 3. molecular biology analysis for ESBL-producing Enterobacteriaceae

Gene	Number of strains
CTX-M	18
SHV	3
TEM	3
CTX-M +TEM	6
CTX-M+ SHV	5

In other studies CTX-M type ESBL are predominant (45% of all E-ESBL+), mainly in *Escherichia coli* (34.5%). The TEM of ESBL was the second predominant type (34.5%), mainly in *Enterobacter aerogenes* (18.6%) and *Klebsiella pneumoniae* (9.4%). SHV ESBL was found mainly in Enterobacter cloacae (7.5%). Several epidemic situations were identified, with CTX-M15 in *Escherichia coli* (21, 22 and 23)

In Tunisia, the first isolate found to produce a CTX-M de type blactamase was an *E. coli* strain recovered from the urine of a surgical patient (8 March 2000). Two months later, other isolates harboring the CTX-M PCR consensus sequence was found in the general medicine ward and subsequently in other wards [24]

In other studies the ESBL was identified as CTX-M-28 by sequencing of PCR products and isoelectric focusing. The ESBL resistance was transferred by a 50 kb plasmid. CTX-M-28 is closely related to CTX-M-15, also the first description of this enzyme was in Tunisia by Ben Achour et al(25)

3.3 THE GENE RESPONSIBLE FOR RESISTANCE TO METHICILLIN

The gene responsible for resistance to Methicillin in our case was mec A detected in the six strains tested Methicillin-resistant. S. aureus (MRSA) (table 3) was considered to be resistant to all b-lactam antibiotics, this bacteria is epidemic in hospitals environments in parts of Europe (e.g.Greece, UK), Japan and USA.

Table 4. Analysis by molecular biology of Staphylococcus aureus Methicillin resistance

Gene	number of strains
Mec A	6

In some areas, 40–60% of hospital S. aureus isolates are MRSA (26) methicillin-resistant have been isolated from hospital wastewaters (Schwartz et al., 2003 (27). Previous studies have shown that S. aureus has a low prevalence in hospital and municipal wastewaters (Shannon et al., 2007) (28).

The mecA gene has been detected both in municipal and in hospital wastewaters (29)

4 CONCLUSION

Bacteria which were sent from provinces indicated that Enterobacteriaceae (42.3%) was prevalent, ESBL Enterobacteriaceae representing 43.6% Followed by *pseudomonas aeurogenosa* and *sthaphylococoque* .

CTX was the most prevalent gene, it represented 51.5% of ESBL Enterobacteriaceae, and mec A The gene responsible for resistance to Methicillin was detected in the six strains tested.

REFERENCES

[1] Archibald L., Phillips L., Monnet D., McGowan JE Jr., Tenover F., Gaynes R., Antimicrobial resistance in isolates from inpatients and outpatients in the United States:increasing importance of the intensive care unit. Clin Infect Dis. 24:211-215 (1997).

[2] Flamm RK., Weaver MK., Thornsberry C., Jones ME., Karlowsky JA., Sahm DF., Factors associated with relative rates of antibiotic resistance in Pseudomonas aeruginosa isolates tested in clinical laboratories in the United States from1999 to 2002., Antimicrob Agents Chemother. 48: 2431-6 (2004).

[3] Rhomberg PR, Jones RN, Sader HS. Results from the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) Programme: report of the 2001 data from 15 United States medicalcentres. Int J Antimicrob Agents. 23:52–9 (2004).

[4] Sader HS, Biedenbach DJ, Jones RN. Global patterns of susceptibility for 21 commonly utilized antimicrobial agents tested against 48, 440 Enterobacteriaceae in the Sentry Antimicrobial Surveillance Program (1997–2001). DiagnMicrobiol Infect Dis. 47:361–4 (2003).

[5] Fridkin SK, Steward CD, Edwards JR. Surveillance of antimicrobial use and antimicrobial resistance in United States hospitals: project ICARE phase 2. Project Intensive Care Antimicrobial Resistance Epidemiology (ICARE) hospitals.Clin Infect Dis. 29:245–52(1999).

[6] Hsueh PR, Teng LJ, Chen CY, Chen WH, Wu Ho S, and Tay Luh K. Pandrug-resistant *Acinetobacter Baumannii* causing nosocomial infections in a university hospital,Taiwan. Emerg Infect Dis.8:827–32(2002).

[7] Hsueh PR, Chen ML, Sun CC, Chen WH, Pan HJ, Yang LS, Emergence of antimicrobial drug resistance of major pathogens causing nosocomial infections at a university hospital in Taiwan, 1981-1999. Emerg Infect Dis. 2002;8:63–8

[8] Hsueh PR, Liu YC, Yang D, Yan JJ, Wu TL, Huang WK, Multicenter surveillance of antimicrobial resistance of major bacterial pathogens in intensive care units in 2000 in Taiwan. Microb Drug Resist. 2001;7:373–82

[9] Karlowsky JA, Jones ME, Thornsberry C, Frielland IR, Sahm DF. Trends in antimicrobial susceptibilities among Enterobacteriaceae isolated from hospitalized patients in the United States from 1998 to 2001. Antimicrob Agents Chemother.47:1672–80(2003).

[10] Nordmann P, Poirel L. Emerging carbapenemases in gram-negative aerobes. ClinMicrobiol Infect;8:321–31(2002).

[11] Van Eldere J. Multicentre surveillance of *Pseudomonas aeruginosa* susceptibility patterns in nosocomial infections. J Antimicrob Chemother;51:347–52(2003).

[12] Archibald L, Phillips L, Monnet D, Mac Gowan JE Jr, Tenover F, Gaynes R. Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. Clin Infect Dis.;24:211-215(1997).

[13] National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A6,6th ed. Wayne, PA: NCCLS (1999).

[14] Thabaut, A. &Meyran, M.. In-vitro activity of tazobactam (YTR 830) and piperacillin combinations against 224 isolates of Pseudomonas aeruginosa harboring known -lactamase. Pathological Biology .39, 361–6 (1990).

[15] Mehtar S. The continuing problem of hospital staphylococci: WhyJChemother (Italy); 6 (Suppl. 4): 25-31(1994)

[16] Emmerson M. Nosocomial staphylococcal outbreaks. Scund J Infect Dis. 93 (Suppl. 1): 47-54 (1994)

[17] (Hryniewicz W. Bacterial resistance in Eastern Europe-selected problems. Stand J Infect Dis. 93 (Suppl.): 32-39 (1994)

[18] Kosmidis J, Polychronopoulou-Karakatsanis C, Milano-Petropoulou D, Mavrogenis N, Xenaki-Kondyli M, Gargalianos P. Staphylococcus infections in hospital: the Greekexperience. J Hasp Infect. 11: 109-115(1988).,

[19] Varaldo PE. Epidemiological study of Staphylococcus aureus strains isolated from clinical material in 24 Italian hospitals. EurJEpidemiol. 2: 208-214 (1986)

[20] Gun H, Ozinel MA, Yenen OS.Methicillin resistance of Staphylococci isolated from clinical specimens. Twk J Sot Microbial. ZO(3-4): 211-215(1992).

[21] C. Giraud-Morin a,*, T. Fosse. Recent evolution and characterization of extended-spectrum beta-lactamase producing enterobacteria in the CHU of Nice (2005–2007) PathologieBiologie. 56 : 417–423 (2008)

[22] Cantón R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, Coque TM. Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe.ClinMicrobiol Infect 2008;14 (Suppl. 1): 144–53.

[23] Lavollay M, Mamlouk K, Frank T, Akpabie A, Burghoffer B, Ben Redjeb S, et al. Clonal dissemination of a CTX-M-15 beta-lactamase-producing Escherichia coli strain in the Paris area, Tunis, and Bangui. Antimicrob. Agents Chemother.50(7):2433–8(2006).

[24] Mamlouk K, Ben Boubaker IB, Gautier V, Vimont S, Picard B, Ben Redjeb S, et al. Emergence and outbreaks of CTX-M b-lactamase-producing Escherichia coli and *Klebsiella pneumoniae* strains in a Tunisian hospital. J ClinMicrobiol.44:4049–56(2006).

[25] N. Ben Achour, P.S. Mercuri, P. Power b, C. Belhadj, M. Ben Moussa, M. Galleni, O. Belhadj. First detection of CTX-M-28 in a Tunisian hospital from a cefotaxime-resistant *Klebsiella pneumoniae* strain PathologieBiologie .57: 343–348(2009)

[26] Grundmann, H., Aires-de-Sousa, M., Boyce, J., Tiemersma, E.,. Emergence and resurgence of methicillin-resistant. Staphylococcus aureus as a public-health threat. Lancet 368(9538), 874–885 (2006)

[27] Schwartz, T., Kohnen, W., Jansen, B., Obst, U., Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms.FEMS Microbiol. Ecol. 43 (3), 325–335(2003)

[28] Shannon, K.E., Lee, D.Y., Trevors, J.T., Beaudette, L.A.,.Application of real-time quantitative PCR for the detection of selected bacterial pathogens during municipal wastewater treatment. Sci. Total Environ. 382 (1), 121–129 (2007)

[29] Volkmann, H., Schwartz, T., Bischoff, P., Kirchen, S., Obst, U.,.Detection of clinically relevant antibiotic-resistance genes in municipal wastewater using real-time PCR (TaqMan). J. Microbiol. Methods. 56 (2), 277–286 (2004).