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VIRULENCE FACTORS AND ANTIMICROBIAL RESISTANCE OF ESCHERICHIA COLI ST131 IN COMMUNITY-ONSET HEALTHCARE-ASSOCIATED INFECTIONS IN SICILY, ITALY

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Abstract

Escherichia coli ST131 is an emerging resistant agent recently called "superbug" in England. This strain is responsible of community-acquired urinary tract infections and nowadays showing increasing resistance to antibiotics like fluoroquinolones and cephalosporins. Survey of virulent bacterial clone is relevant to control its spreading in community.

We aim to assess the circulation of resistant clones Escherichia coli ST131 outside of the hospital to prompt control of outbreak in our geographical area.

We selected 105 *E. coli* resistant isolates from community-acquired urinary infections and performed a multiplex PCR to evaluate if they belonged to the ST131 type. We investigated their set of virulence factors; in particular, *kpsMII, papA, sfaS, focG, iutA, papC, hlyD* and *afa* genes, and finally, we evaluated beta lactamases genes and quinolone resistance determinants.

E. coli ST 131 clone was present in 66.6% of our isolates and showed positivity to a wide range of resistance genes, in particular $bla_{CTX\text{-}M\text{-}15}$ among beta lactamases and plasmid-related quinolone resistance genes (*qnrA*, *qnrS* and *aac (6')-Ib-cr*). Moreover, 81% of the strains showed positivity to at least one of the virulence factor genes.

Our results suggested a high presence of *E. coli* ST131 in community. We suggest antibiotic stewardship for outpatient clinicians and facilities to contain the spread of "superbug" agents.

Keywords: *Escherichia coli*, urinary tract infections, antibiotics, fluoroquinolones, cephalosporins

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Introduction

Antibiotic resistance stands nowadays as one of the most alarming health problems not only inside hospitals, but also in community [1-2]. This ancient bacterium [3] is recently become attractive because resistant to three or more different drug classes at the same time are considerate "Multi Drug Resistant" (MDR) bacteria [4-5]. *Escherichia coli* is the primary causative agent for community-acquired urinary tract infections (UTI), whose outcome can be complicated by increasing of antimicrobial resistance. *E. coli* shows resistance mainly against fluoroquinolones, beta-lactams and third-generation cephalosporins. *E. coli* ST131 is one of the most virulent bacterial clones: it usually carries Extended Spectrum Beta-Lactamases (ESBL), like the ones belonging to $bla_{CTX\text{-}M}$ family, and it is now spreading in community environment [5-6], causing extra-intestinal infections. Many Virulence Factors (VFs) can help *E. coli* to induce infections in hosts: they usually allow pathogen to invade host tissues, to trigger an inflammatory response, and to escape immunity response [7-8]; they may include adhesins (P and S fimbriae, F1C), toxins (haemolysins), group II and III capsules, siderophores (aerobactin system) and invasins [9-11]. Antimicrobial resistance, once confined inside hospital ramparts, has now spread in community too, thus becoming an increasing threat in public health [8,10-12]. Fluoroquinolone resistance in *E. coli* strains is due *qnrA*, *qnrB*, *qnrS* and *aac (6')-Ib-cr* genes particularly linked to ST131 *E. coli* clone.

The increasing use of these antimicrobial molecules in therapies against urinary tract infections could have contributed to ST131 broad diffusion [5,8]. Cefalosporin resistance, while, is mediated by Extended Spectrum Beta-Lactamases (ESBL) production, involving $bla_{CTx\text{-}m}$, bla_{OXA}, bla_{TEM} and bla_{SHV} genes, which confer resistance to penicillin, broad spectrum cephalosporins and monobactams [13]. $bla_{CTX\text{-}M}$ appears to be now the most common ESBL in *Enterobacteriaceae* like *E. coli*; there are a lot of genetic variants forming $bla_{CTX\text{-}M}$ family. Among these variants, while $bla_{CTX\text{-}M\ \text{-}15}$ seems to be common in human pathogens, thanks to the diffusion of ST131 pandemic clone [14], $bla_{CTX\text{-}M\ \text{-}1}$ can be found more often in various animal microbiota, mainly birds, poultry, and both wild and breeding mammals [9-15]

The object of this study was to describe *E. coli* clinical isolates from adults patients with UTI in a community in Sicily, Mediterranean region of Southern Italy.

Materials and methods

Strains. 105 *E. coli* resistance to Norfloxacin, Levofloxacin and Ciprofloxacin, were collected from three different clinical laboratories located in Palermo, Sicily, between April 2014 and December 2015. All *E. coli* strains were isolated from urinary samples.

Strain identification and antibiotic susceptibility were done by the Vitek automated system as previously reported [16-19]

DNA extraction. Bacterial strains, stored at -80°C in Brain Heart Infusion Broth (BD) with 10% glycerol were inoculated on Columbia Sheep Blood Agar and incubated at 37°C for 18h. A single colony was suspended in 200 µl sterile bi-distilled water and subjected to DNA extraction protocol according to High Pure PCR Template Preparation Kit (Roche) procedure instructions [20].

ST131 type assignment. To evaluate the circulation of ST 131 group, we performed a Multiplex PCR for *mdh* and *gyrB* genes, according to James R. Johnson model, which examines certain SNPs associated to these two genes. ST131 positivity is showed by two amplicons, whose length is respectively 275 bp and 132 bp [10]. Reactions were conducted in 25 µl PCR tubes, each one containing Green GoTaq® Flexi Buffer 1 X, MgCl2 4 mM, each dNTPs with a final concentration of 0,8 mM, each primer with a final concentration of 0.75 mM and 1,25 U of GoTaq® Flexi DNA Polymerase (Promega). PCR reactions require an initial denaturation at 94 °C for 5 minutes, then 28 cycles with 30" 94°C denaturation, 30" at 65 °C annealing, 30" at 68 °C elongation and a final 3 minutes extension step at 72 °C.

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Plasmid-mediated quinolone resistance (PMQR) *aac*, *qnrA*, *qnrB* and *qnrS* genes were investigated according to Chi Hye Park model [15]. Each 25 µl PCR tube contained Green GoTaq® Flexi Buffers 1X, 1,5 mM MgCl2, 20 mM of each primer, 2,5 mM of each dNTP, 1 U of GoTaq® Flexi DNA Polymerase (Promega) and 1,5 µl of DNA. PCR reactions were performed with an initial denaturation for 4' at 95°C, followed by 35 cycles with 45" denaturation at 94°c, 45" annealing at 55°C, 45" polymerization at 72°C and a final extension step at 72°C for 10'. Similar conditions applied to *qnrA*, *qnrB* and *qnrS* amplification, only with different annealing temperatures (53°C for *qnrA* and 57°C for *qnrB* and *qnrS*).

Cephalosporin resistance. Beta-lactamases such as OXA, TEM, SHV (Multi TSO) and $bla_{CTX\text{-}M}$ -like ESBL were investigated with Multiplex PCR reactions, according to Dallenne model [18]. Multi TSO PCR was conducted in 25 µl tubes, each containing Green GoTaq® Flexi Buffers 1X, 1,5 mM of MgCl2, 2,5 mM of each dNTPs, 1 U di GoTaq® Flexi DNA Polymerase (Promega) and 0,4 pmol/µL of each primer. Reactions were conducted with an initial 94°C denaturation for 10', followed by 30 cycles with 94°C denaturation for 40'' 60°C annealing for 40'', 72°C extension for 1' plus a final extension step for 7' at 72°C. $bla_{CTX\text{-}M}$ was investigated with a single-step PCR, following Pagani model [21]. Each 25 µl tube contained Green GoTaq® Flexi Buffers 1X, 1,5 mM of MgCl2, 20 mM of each, 2,5 mM of each dNTP, 1 U of GoTaq® Flexi DNA Polymerase (Promega) and 1,5 µl of sample DNA. PCR was performed with an initial 94 °C denaturation for 4 minutes, 35 cycles with 94 °C denaturation for 45", 50 °C annealing for 1 minute, 72 °C elongation for 45" and a final 72 °C extension for 10 minutes.

Virulence factors. Two different Multiplex PCR were performed, as proposed by Lotte Jakobsen; one for *kpsMII* (group II capsule), *papA* (pilus-associated protein A), *sfaS* (S-fimbrial adhesine) and *focG* (F1C fimbriae protein) genes, the other one for *hlyD (haemolysin D), afa (an afimbrial adhesine) iutA (an iron-chelating protein) and papC (Pilus-associated protein C) genes* [7]. Each 25 µl PCR tube contained Green GoTaq® Flexi Buffers 1X, MgCl2 1,5 mM, dNTP 2,5 mM, 20 µM of each primer, 1,25 U GoTaq® Flexi DNA Polymerase (Promega) and 1,5 µl of DNA. Reactions were performed with an initial denaturation at 94°C for 4', followed by 24 cycles with 30'' denaturation at 94°C, per 30'' annealing at 61°C and 1' extension at 68°C, with a final extension step at 72°C for 10'. Three bacterial strains were used as positive control: *E. coli* RS218 strain, *E. coli* V27 strain and *E. coli* 2H16 strain.

Results

In the present study, we analyzed 105 quinolone-resistant *E.coli* isolated from UTI. We found that *E. coli* ST131 strains were predominant (66.6% ST131 positive; 33.3% non-ST131) among our samples.

The genes *aac(6')-Ib-cr*, *qnrA*, *qnrB* and *qnrS* correlated to quinolone resistance were found in only 56,2% of our isolates. In particular, *aac(6')-Ib-cr* and *qnrA* genes both appeared in 27 of 105 isolates; while 5 of 105 isolates were positive to *qnrS*. No strain showed positivity to *qnrB* gene.

About to relation between PQMR genes and ST131 strains, while *aac* and *qnrA* were equally present in both ST131 and non-ST131 strains, *qnrS* presence seems to be higher in non-ST131 strains (Figure.1)

We furthermore analysed for cephalosporin resistance determinants, and specifically for $bla_{\text{-}oxa}$ bla_{SHV}, $bla\text{-}_{TEM}$ genes. 67.6% of our *E. coli* isolates were positive to at least one of these genes, 71 strains out of 105. Their association looked quite infrequent, with only 4 strains showing $bla_{\text{-}oxa}$ and $bla_{\text{-}SHV}$ together, and 3 strains showing $bla_{\text{-}oxa}$ and $bla\text{-}_{TEM}$ together. No strain showed an association between $bla_{\text{-}SHV}$ and $bla\text{-}_{TEM}$.

Cephalosporin resistance genes have been then related to ST131 and non-ST131 types. Interestingly, $bla_{\text{-}oxa}$ and $bla_{\text{-}TEM}$ seem to be more common in ST131 strains, while on the other hand $bla_{\text{-}SHV}$ seems to be significantly related to non-ST131 strains. In fact, among the 35 positive strains to $bla_{\text{-}oxa}$, 28 belong to ST131 and 7 to non-ST131 type; $bla_{\text{-}SHV}$ showed positivity on 7

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non-ST131 strains and 2 ST131, and *bla*$_{-TEM}$ appeared positive in 16 ST131 strains and 11 non-ST131 (Figure 2).

We therefore investigated for presence of virulence factors (VFs) in our isolates by using two different multiplex PCRs, each one searching for 4 VFs genes. First multiplex PCR was used to research *hlyD*, *afa, iutA* and *papC* genes (Figure 3*a*), 86.7% of our bacterial strains carries at least one of these VFs genes. Three strains were positive for *hlyD* and *afa*; 10 strains for *iutA* and *papC* together, 5 strains for *afa* and *iutA* genes but no strain showed association between *hlyD* and *iutA* genes. The most common association appeared to be *hlyD* + *iutA* + *papC*, that we found in 17 out of 105 strains (16.2%). The Multiplex PCR was used to investigate about *kpsMII*, *papA, sfaS,* and *focG* genes presence (Figure 3*b*), 81% of our isolates showed posititvity to at least one of them; the most common association was the one between *kpsMII* e *focG*, which we found in 30 strains (28.6%); in addition, all strains carrying *papA* genes were positive to *focG* gene too.

hlyD and *papA* genes were present only in ST131 strains, while *iutA* appeared to be more common on non-ST131. The remaining VFs genes appeared to be equally distributed on ST131 and non-ST131 isolates.

Finally, we analysed for ESBL *bla*$_{-CTX-M}$ gene. We found out that 49 of our samples (46,7%) carried *bla*$_{-CTX-M}$ genes and, among these, 35 belonged to ST131. All positive samples were submitted for sequencing, showing *bla*$_{-CTX-M\ -15}$ variant for ST131 strains, and *bla*$_{-CTX-M\ 1}$ variant for the remaining 14 non-ST131 samples.

Discussion

Antimicrobial resistance has become a public health priority worldwide. The antibiotic resistance situation is not uniform in EU, and in general higher resistance frequencies are reported by countries in eastern and southern Europe [23]. Italy is one of the European countries with increasing spread of antimicrobial-resistant microorganisms especially in adult and Pediatric Intensive Care Units [24-27] The clinical management infections due to multidrugs gram negative microorganism is difficult and nowadays under debates [28-30]

Knowledge of a recent Escherichia coli associated outbreak prompted interviewers to ask about baking and spreading of new types of E. coli called "superbug" England [31] prompted us to investigate the prevalence of this strain in our geographic area.

In the present study, we focused attention on 105 *E. coli* strains isolated from patients with UTI, most of our samples came from elderly people living in nursing homes or rehabilitation centres.

E. coli strains of ST131 were found to constitute the majority (66,6%) of quinolone resistant and ESBL producers. These data are in accordance with previous studies illustrating the emerging of resistant ST131 *E. coli* clone [31]; and they are quickly diffusing and adapting both to hospital and community environments [32,33].

The *acc(6')-Ib-cr* and *qnr-A* genes, which contribute to quinolone resistance, were found in the same percentage of ST131 and non-ST131 strains, while the gene *qnr-S* were associated more frequently with non-ST131 strains. The latter gene, is still less diffuse in E. coli, and recently *qnr-S* has been spreading within *E. coli* strains in other Mediterranean area like Greece [24]; noticeably, this resistance gene appeared to be frequent in pets and courtyard animals and in Italian studies suggesting the role of food chain in antibiotic resistance transmission to humans [8].

The genes *aac(6')-Ib-cr*, *qnrA*, *qnrB* and *qnrS* correlated to quinolone resistance were found in only 56,2%, we hypnotize that the strains of *E. coli* in which we didn't found any PMQR genes, could carry chromosome-located resistance genes, like efflux pumps, or point mutations in *gyrA* or *parC* genes. We therefore focused on ESBL genes, like. In 67.6% of our samples we found at least one of *bla*$_{OXA}$, *bla*$_{SHV}$, *bla*$_{TEM}$ and *bla*$_{CTX-M}$ genes. While usually ST131 type appears to be the greatest ESBL producer, we found that SHV is frequent associate to non-ST131 type, as shown in Figure 3*b*.

Nearly half of our isolates belong to ST131 strains showed *bla*$_{-CTX-M\ -15}$, the most common

ESBL in human *E. coli*, while *bla-CTX-M -1*, was found in chicken and pigs, occurred among non-ST131 strains, as confirmed elsewhere [35]. Pets and breeding animals are in fact the main reservoir for MDR genes, which spread by food, water and animal dejections [5, 8,11,35].

FocG gene present in 81 strains*, iutA* gene present in 88 *E. coli* analysed *and kpsMII* gene positive in 34 of our samples were considered to be the most frequent VFs among our strains suggesting a more predominant role for this gene in extra-intestinal pathogen. Finally the presence of *papC* gene in 34 strains and *papA* in 4 strains can support the hypothesis that resistant bacteria could come from breeding animals. Overall, our bacterial strains didn't own a heavy set of VFs genes; it has been underlined the relationship between quinolone resistance and VFs gene reduction, due to the loss of VFs genes or, conversely, to the fact that less virulent strains are more prone to acquire resistance determinants [4,5,35].

A separate consideration deserves so-called "frailty patients" that in the community are children, the elderly or immunocompromised subjects such as HIV positive and cancer patients [36-41].

In this category, the spread of resistant pathogens could trigger outbreaks as observed in Canada. For this reason, nowadays educational programs are being proposed in Italy to contain old and new infectious agents through vaccination especially in extreme ages such as children and the elderly [41-45]

In conclusion, we can assume that antimicrobial stewardship requirements to be comprehensive of the involvement of paediatricians and family physicians to control the antibiotic abuse.

References

1. A.P. Magiorakos, A. Srinivasanb, R.B. Careyb, Y. Carmelic, M.E. Falagasd, C.G. Giskef, S. Harbarthg, J.F. Hindlerh, G. Kahlmeteri, B. Olsson-Liljequistj, D.L. Patersonk, L.B. Ricel, J. Stellingm, M.J. Struelensa, A. Vatopoulosn, J.T. Weberb, D.L. Monnet: " Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance". Clinical Microbiology and Infection, Volume 18, Issue 3, March 2012: 268–281.
2. Rogers BA, Sidjabat HE, Paterson DL (2011) Escherichia coli O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother. 66: 1–14.
3. Chiarini A, Giammanco A.Relationships of adhesive properties to virulence in gram-negative bacteria. In vitro" interactions between "E. coli" strains and HELA cultures.Ann Sclavo. 1978 ;20(3):337-49.
4. Adams-Sapper S, Diep BA, Perdreau-Remington F, Riley LW. 2013. Clonal composition and community clustering of drug-susceptible and -resistant Escherichia coli isolates from bloodstream infections. Antimicrob Agents Chemother 57: 490–497
5. Giufre M, Accogli M, Farina C, Giammanco A, Pecile P, Cerquetti M. Predominance of the fimH30 subclone among multidrug-resistant Escherichia coli strains belonging to sequence type 131 in Italy. J Infect Dis. 2014 Feb 15;209(4):629-30.
6. Giammanco A, Maggio M, Giammanco G, Morelli R, Minelli F, Scheutz F, Caprioli A. Characteristics of Escherichia coli strains belonging to enteropathogenic E. coli serogroups isolated in Italy from children with diarrhea. J Clin Microbiol. 1996 Mar;34(3):689-94
7. Giammanco A, Calà C, Fasciana T, Dowzicky MJ.Global Assessment of the Activity of Tigecycline against Multidrug-Resistant Gram-Negative Pathogens between 2004 and 2014 as Part of the Tigecycline Evaluation and Surveillance Trial. mSphere. 2017 Jan 18;2(1). pii: e00310-16.
8. Lotte Jakobsen, Daniel J. Spangholm, Karl Pedersen, Lars B. Jensen, Hanne-Dorthe

Emborg, Yvonne Agersø, Frank M. Aarestrup, Anette M. Hammerum, Niels Frimodt-Møller.Broiler "Chickens, broiler chicken meat, pigs and pork as sources of ExPEC related virulence genes and resistance in Escherichia coli isolates from community-dwelling humans and UTI patients", International Journal of Food Microbiology 142 (2010) 264–272.

9. Ghodousi A, Bonura C, Di Carlo P, van Leeuwen WB, Mammina C.Extraintestinal pathogenic Escherichia coli sequence type 131 H30-R and H30-Rx subclones in retail chicken meat, Italy. Int J Food Microbiol. 2016 Jul 2;228:10-3
10. Caprioli A, Pezzella C, Morelli R, Giammanco A, Arista S, Crotti D, Facchini M, Guglielmetti P, Piersimoni C, Luzzi I. Enteropathogens associated with childhood diarrhea in Italy. The Italian Study Group on Gastrointestinal Infections. Pediatr Infect Dis J. 1996 Oct;15(10):876-83.
11. Jonas Bonnedahl, Jorge Hernandez, Johan Stedt, Jonas Waldenström, Björn Olsen e Mirva DrobniExtended-Spectrum β-Lactamases in Escherichia coli and Klebsiella pneumoniae in Gulls, Alaska, USA. Emerging Infectious Diseases Vol. 20, No. 5, May 2014.
12. Poeta P, Radhouani H, Pinto L, Martinho A, Rego V, Rodrigues R, Gonçalves A, Rodrigues J, Estepa V, Torres C, Igrejas G. Wild boars as reservoirs of extended-spectrum beta-lactamase (ESBL) producing Escherichia coli of different phylogenetic groups. J Basic Microbiol. 2009 Dec;49(6):584-8. doi: 10.1002/jobm.200900066.
13. Chi Hye Park, Ari Robicsek, George A. Jacoby, Daniel Sahm e David C. Hooper. Prevalence in the United States of aac(6)-Ib-cr Encoding a Ciprofloxacin-Modifying Enzyme. Antimicrobial agents and chemotherapy 2006, Vol. 50, No. 11. p. 3953–3955.
14. A.P. Magiorakos, A. Srinivasanb, R.B. Careyb, Y. Carmelic, M.E. Falagasd, C.G. Giskef, S. Harbarthg, J.F. Hindlerh, G. Kahlmeteri, B. Olsson-Liljequistj, D.L. Patersonk, L.B. Ricel, J. Stellingm, M.J. Struelensa, A. Vatopoulosn, J.T. Weberb, D.L. Monnet: " Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance". Clinical Microbiology and Infection, Volume 18, Issue 3, March 2012: 268–281
15. Chi Hye Park, Ari Robicsek, George A. Jacoby, Daniel Sahm e David C. Hooper. Prevalence in the United States of aac(6)-Ib-cr Encoding a Ciprofloxacin-Modifying Enzyme. Antimicrobial agents and chemotherapy 2006, Vol. 50, No. 11. p. 3953–3955.
16. Di Carlo P, Pantuso G, Cusimano A, D'Arpa F, Giammanco A, Gulotta G, Latteri AM, Madonia S, Salamone G, Mammina C. Two cases of monomicrobial intraabdominal abscesses due to KPC—3 Klebsiella pneumoniae ST258 clone. BMC Gastroenterol. 2011 Sep 30;11:103. doi: 10.1186/1471-230X-11-103.
17. Djoudi F, Bonura C, Benallaoua S, Touati A, Touati D, Aleo A, Cala C, Fasciana T, Mammina C. Panton-Valentine leukocidin positive sequence type 80 methicillin-resistant Staphylococcus aureus carrying a staphylococcal cassette chromosome mec type IVc is dominant in neonates and children in an Algiers hospital. New Microbiol. 2013 Jan;36(1):49-55. Epub 2013 Jan 1
18. Hojabri Z, Pajand O, Bonura C, Aleo A, Giammanco A, Mammina C. Molecular epidemiology of Acinetobacter baumannii in Iran: endemic and epidemic spread of multiresistant isolates. J Antimicrob Chemother. 2014 Sep;69(9):2383-7. doi: 10.1093/jac/dku045. Epub 2014 Feb 20.
19. Fasciana T, Calà C, Bonura C, Di Carlo E, Matranga D, Scarpulla G, Manganaro M, Camilleri S, Giammanco A. Resistance to clarithromycin and genotypes in Helicobacter pylori strains isolated in

Sicily. J Med Microbiol. 2015 Nov;64(11):1408-14.
20. James R. Johnson and Adam L. Stell :" Extended Virulence Genotypes of Escherichia coli Strains from Patients with Urosepsis in Relation to Phylogeny and Host Compromise". J Infect Dis. (2000) 181 (1): 261-272.
21. Laura Pagani, Emanuela Dell'Amico, Roberta Migliavacca, Marco Maria D'Andrea, Ernesto Giacobone, Gianfranco Amicosante, Egidio Romero e Gian Maria Rossolini. Multiple CTX-M-Type Extended-Spectrum β-Lactamases in Nosocomial Isolates of Enterobacteriaceae from a Hospital in Northern Italy. . J Antimicrob Chemother 2003, Vol. 41, No. 9. p. 4264–4269.
22. Dallenne, Caroline, et al. "Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae." Journal of Antimicrobial Chemotherapy 65.3 (2010): 490-495.
23. European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2012. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net)Stockholm: ECDC; 2013.1: Barberi G, De Cola MC, Dell'Utri C, Melardi S, Alagna B, Bramanti P, Cascio A. Antimicrobial consumption and antimicrobial resistance: a snapshot of an Italian neuromuscular rehabilitation center. New Microbiol. 2017 Apr;40(2):119-129.
24. Mammina C, Calà C, Bonura C, Di Carlo P, Aleo A, Fasciana T, Giammanco A; EPI-MRSA Working Group. Polyclonal non multiresistant methicillin resistant Staphylococcus aureus isolates from clinical cases of infection occurring in Palermo, Italy, during a one-year surveillance period. Ann Clin Microbiol Antimicrob. 2012 Jun 19;11:17
25. Mammina C, Palma DM, Bonura C, Aleo A, Fasciana T, Sodano C, Saporito MA, Verde MS, Calà C, Cracchiolo AN, Tetamo R. Epidemiology and clonality of carbapenem-resistant Acinetobacter baumannii from an intensive care unit in Palermo, Italy. BMC Res Notes. 2012 Jul 20;5:365.
26. Mammina C, Bonura C, Aleo A, Calà C, Caputo G, Cataldo MC, Di Benedetto A, Distefano S, Fasciana T, Labisi M, Sodano C, Palma DM, Giammanco A. Characterization of Acinetobacter baumannii from intensive care units and home care patients in Palermo, Italy. Clin Microbiol Infect. 2011 Nov;17(11):E12-5
27. Iaria C, Stassi G, Costa GB, Biondo C, Gerace E, Noto A, Spinella SG, David A, Cascio A. Outbreak of multi-resistant Corynebacterium striatum infection in an Italian general intensive care unit. J Hosp Infect. 2007 Sep;67(1):102-4.
28. Di Carlo P, Vitale F, O'Súilleabháin C, Casuccio A. Management of Intra-abdominal Infections due to Carbapenemase-Producing Organisms. Curr Infect Dis Rep. 2014 Oct;16(10):428.
29. Cascio A, Mezzatesta ML, Odierna A, Di Bernardo F, Barberi G, Iaria C, Stefani S, Giordano S. Extended-spectrum beta-lactamase-producing and carbapenemase-producing Enterobacter cloacae ventriculitis successfully treated with intraventricular colistin. Int J Infect Dis. 2014 Mar;20:66-7.
30. Cascio A, Conti A, Sinardi L, Iaria C, Angileri FF, Stassi G, David T, Versaci A, Iaria M, David A. Post-neurosurgical multidrug-resistant Acinetobacter baumannii meningitis successfully treated with intrathecal colistin. A new case and a systematic review of the literature. Int J Infect Dis. 2010 Jul;14(7):e572-9.
31. Kallonen T, Brodrick HJ, Harris SR, Corander J,3,4, Brown NM,6, Martin V, Peacock SJ, Parkhill J. Systematic longitudinal survey of invasive Escherichia coli in England demonstrates a stable population structure only transiently disturbed by the emergence of ST131. Genome Res. 2017 Jul 18. doi:

10.1101/gr.216606.116. [Epub ahead of print]
32. Cheol-In Kang, Min Kyeong Cha, So Hyun Kim, Kwan Soo Ko, Yu Mi Wi, Doo Ryeon Chung, Kyong Ran Peck, Nam Yong Lee e Jae-Hoon Song. Clinical and Molecular Epidemiology of Community-Onset Bacteremia Caused by Extended-Spectrum β-Lactamase- Producing Escherichia coli over a 6-Year Period. Infectious Diseases, Microbiology & Parasitology. Korean Med Sci 2013; 28: 998-1004.
33. Johnson J, Nicolas-Chanoine M-H, DebRoy C, Castanheira M, Robicsek A, Hansen G, et al. Comparison of Escherichia coli ST131 pulsotypes, by epidemiologic traits, 1967–2009. Emerg Infect Dis. 2012;18:598–607.
34. Elisabet Guiral, Jordi Bosch, Jordi Vila and Sara M. Soto: Prevalence of Escherichia coli among samples collected from the genital tract in pregnant and nonpregnant women: relationship with virulence. FEMS Microbiology Letters, Volume 314, Issue 2, pages 170–173, January 2011.
35. Meyer E, Gastmeier P, Kola A, Schwab F. Pet animals and foreign travel are risk factors for colonisation with extended-spectrum β-lactamase-producing Escherichia coli. Infection. 2012 Dec;40(6):685-7.
36. Marin D, Cappabianca S, Serra N, Sica A, Lassandro F, D'Angelo R, La Porta M, Fiore F, Somma F. CT Appearance of Hepatocellular Carcinoma after Locoregional Treatments: A Comprehensive Review. Gastroenterol Res Pract. 2015;2015:670965
37. Reginelli A, Vanzulli A, Sgrazzutti C, Caschera L, Serra N, Raucci A, Urraro F, Cappabianca S. Vascular microinvasion from hepatocellular carcinoma: CT findings and pathologic correlation for the best therapeutic strategies. Med Oncol. 2017 May;34(5):93.
38. Li Vecchi V, Soresi M, Colomba C, Mazzola G, Colletti P, Mineo M, Di Carlo P, La Spada E, Vizzini G, Montalto G. Transient elastography: a non-invasive tool for assessing liver fibrosis in HIV/HCV patients. World J Gastroenterol. 2010 Nov 7;16(41):5225-32.
39. Colomba C, Saporito L, Vitale F, Reale S, Vitale G, Casuccio A, Tolomeo M, Maranto D, Rubino R, Di Carlo P, Titone L.Cryptic Leishmania infantum infection in Italian HIV infected patients. BMC Infect Dis. 2009 Dec 10;9:199
40. Colomba C, Rubino R, Siracusa L, Lalicata F, Trizzino M, Titone L, Tolomeo M. Disseminated tuberculosis in a patient treated with a JAK2 selective inhibitor: a case report BMC Res Notes. 2012 Oct 5;5:552.
41. Giammanco A, Nardone A, Pebody R, Kafatos G, Andrews N, Chiarini A, Taormina S, de Ory F, Prosenc K, Krize B, Hallander H, Ljungman M, Marva E, Tsakris A, O'Flanagan D, Schneider F, Griskevicius A, Vranckx R, Karacs I.European Sero-Epidemiology Network 2: standardisation of immunoassay results for pertussis requires homogeneity in the antigenic preparations. Vaccine. 2008 Aug 18;26(35):4486-93.
42. Gabutti G, Bergamini M, Bonanni P, Guido M, Fenoglio D, Giammanco A, Sindoni L, Zotti C, Boddi V, Bamfi F, Severini R, Bechini A, Boccalini S, Crovari P; Collaborative Group for the Study of Pertussis.Assessment of humoral and cell-mediated immunity against Bordetella pertussis in adolescent, adult, and senior subjects in Italy. Epidemiol Infect. 2008 Nov;136(11):1576-84.
43. Giammanco A, Taormina S, Chiarini A, Dardanoni G, Stefanelli P, Salmaso S, Mastrantonio P. Analogous IgG subclass response to pertussis toxin in vaccinated children, healthy or affected by whooping cough. Vaccine. 2003 May 16;21(17-18):1924-31.

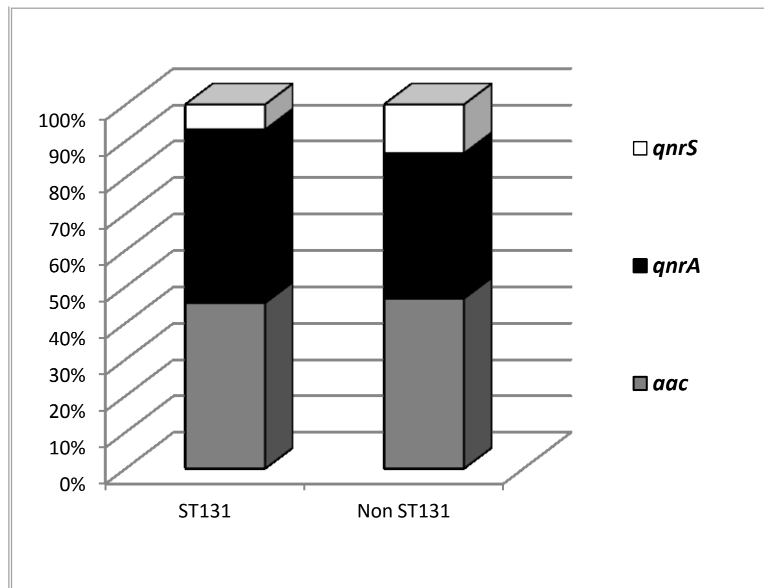

Figure 1. Percentage of PQMR genes.

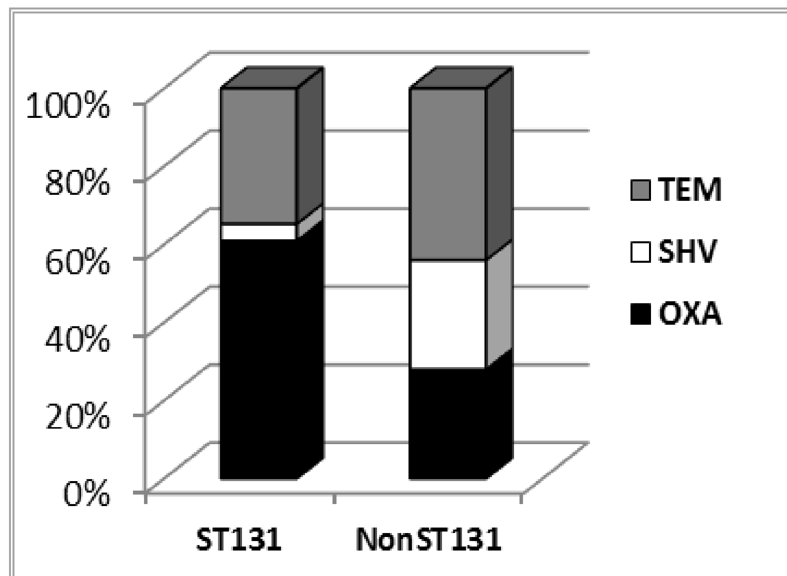

Figure 2. Distribution of ESBL genes (*bla-$_{oxa}$ bla$_{SHV,}$ bla-$_{TEM}$*) among ST131 and non-ST131 isolates.

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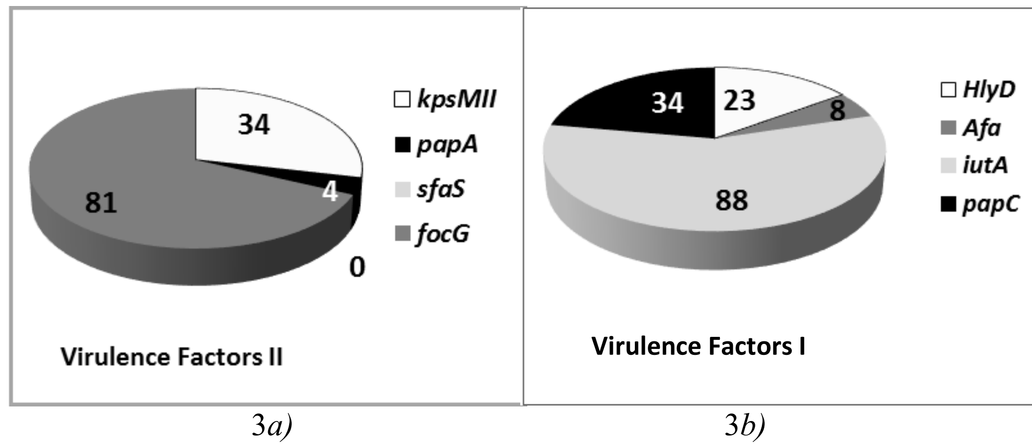

3*a)* 3*b)*

Figure 3. Virulence Factors founded in our isolate; *a)* First multiplex PCR was used to research *hlyD*, *afa, iutA* and *papC* genes presence; *b)* The Multiplex PCR was used to investigate about *kpsMII*, *papA, sfaS,* and *focG* genes presence

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