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Expanding PVL positive MRSA healthcare-associated infections outbreak in a newborns unit, caused by hospital hygiene shortcomings

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Abstract

A prolonged outbreak of Healthcare-Associated Infections (HCAIs) evolved since December 2013, in a Newborns Unit from Hospital A, sited in the North-Eastern development region, Romania. A first cluster consisted of 19 cases, of which 18 infections in newborns and 1 labour infectious complication in a mother. Except for five cases declared and treated in the Neonatology Unit as hospital-acquired infections, the other cases were discharged and further required rehospitalisation and treatment.

Eight of these innitialy discharged cases were readmitted to the Pediatric Surgery Unit and two others to the Pediatrics Unit of Hospital B, while three others were readmitted to three hospitals: one to the Pediatrics Unit of Hospital C, and other two to Hospital A and Hospital D, respectively. The mother with the labour infectious complication was readmitted to the Gynecology Unit of the Hospital A.

A number of fifteen Staphylococcus aureus (S. aureus) strains isolated from the HCAI first episode and 8 strains from 7 HCWs were received by „Cantacuzino" Institute, Nosocomial Infections and Antibiotic Resistance Laboratory from the County Public Health Directorate, for confirmation and molecular typing.

After a first round of interventions for infection control, a second episode bursted in Hospital A and our laboratory received six other S. aureus isolates from newborns, hospital environment, and HCWs.

Public Health interventions based on epidemiologic data and molecular microbiology results were finally successful. The evolution of all cases was favorable.

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An important factor favoring the outbreak was the moving of the Birth Unit of Hospital A to an innapropriate location for an 18-month interval, more than innitially estimated, in relation to rehabilitation of the ward.

We considered to report this episode taking into account the unusual evolution, the risk of multiresistant bacterial strains spreading, and multiple unwanted consequences caused by shortcomings in providing appropriate hygiene conditions.

***Keywords**:MRSA, lukS/F-PV genes, spa type t008, HCAI outbreak, newborns unit, hospital hygiene*

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Introduction

According to the World Health Organization (WHO), "Healthcare-Associated Infection (HCAI), also referred to as "nosocomial" or "hospital" infection, is an infection occurring in a patient during the process of care in a hospital or other healthcare facility which was not present or incubating at the time of admission. HCAI can affect patients in any type of setting where they receive care and can also appear after discharge. Furthermore, they include occupational infections among staff." Particular risk factors like low birth weight, gestational age, length of stay pending on different associated pathologies and instrumentation are recognized in Newborn Intensive Care Units (NICUs) (1).

Newborn skin layer microstructure differs from adult skin, and particular pre-term, post-term and term skin changes are common in the first month of life adaptation period. There are physiological dermatological phenomena that occur, including newborns erythema, milia papules, miliaria, neonatal acne, sebaceous hyperplasia, non-infectious vesicopustular lesions, etc. The newborn skin is very susceptible to infection (2).

Descriptive epidemiology of the first Newborns Unit outbreak

Hospital A has 460 beds and functions in two different locations at 2 km from each other.

The Hospital Maternity is a medium sized one, with a Neonatology Unit of 30 beds and an Obstetrics/ Gynaecology Unit with 50 beds. It is sited in a monoblock building, dating since 1983, and it covers the second-largest territory of the county and a population of 155061 people, of which 61495 in the urban area and 93566 in the rural area.

In the August 2012 - November 2013 interval, the Maternity was refurbished in the frame of a World Bank Program. During this interval, the Maternity functioned in the space of the Gynaecology ward, which was temporarily merged with the Surgery ward. The operating license was delivered by the County Public Health Authority under strict clauses, because of surface, space partitioning, and problems with functional flows. The Modernized Maternity reopened on December 4th 2013.

Sporadic detection of *S. aureus* on environment surfaces and newborns skin was reported with the occasion of the 9 salubrity tests performed during the August 2012 - November 2013 interval.

The incidence of nosocomial infections reported in the Newborns Unit was low (0.8-0.9% of hospital discharged newborns). However, in the last three years all infections have been skin infections, which may suggest some problems in newborn care procedures, despite the fact that specific procedures are in place for both healthy and premature newborn care, hand washing and antisepsis, cleaning and disinfection and TB vaccination.

A first cluster of HCAI evolving in Hospital A consisted of 19 cases, of which 18 infections in

newborns and 1 labour infectious complication in a mother. On December 16th 2013, the epidemiologist from Hospital B, which is the County Emergency Hospital, reported the admission to the Paediatric Surgery Unit of 3 newborns (cases 2, 3, 4), aged 12 to 17 days, all born in Hospital A. All of them presented with skin infections, required surgery and were diagnosed with Methicillin-Resistant *S. aureus* (MRSA) infection. The retrospective study of patients records revealed another previous case (case 1), aged 17 days, admitted to the same ward on November 19th, with a clinical diagnosis of left breast abscess.

On December 17th 2013, a team coordinated by the senior epidemiologist from the County Public Health Directorate point of work, sited in Hospital A city started the epidemiological investigation and took the first microbiological samples from the Maternity.

A major risk factor for infections in newborns was considered to be the lack of warm water caused by the breakdown of a water pump in the very day of moving to the new location, which was not resolved until after two weeks. During this interval, the toilet of the newborns was accomplished by using wet wipes.

On December 17th 2013, two other newborns coming from the Hospital A Maternity with the diagnosis of breast abscess/ staphylococcal skin infection, were admitted to Hospital B – Paediatric Surgery ward (cases 5 and 6). The expansion of the outbreak led the local public health authorities to decide on the temporary closure of Hospital A Maternity starting December 18th 2013. On the same day, two new cases (7 and 11) were hospitalized in Hospital B with staphylococcal skin infection. The decision was communicated to the Ministry of Health and local authorities.

A number of 8 mothers and their healthy newborns were then transferred from Hospital A to Hospital B, being sampled for detection of contamination/ colonisation with *S. aureus*. Cases with positive results for MRSA received prophylactic antimicrobial therapy, according to antimicrobial susceptibility testing results. Finding of MRSA colonisation in all transferred newborns indicated a strong *S. aureus* dissemination in Hospital A Maternity. Infection cases with onset in the new location led the investigation team to the hypothesis that the source of infection would be represented by healthcare workers (HCWs), which was confirmed by the high number of *S. aureus* nose or pharyngeal carriers in Maternity staff members, i.e. 22 persons (35.5%).

On Public Health County Authority request, Hospital C, sited in the same city as Hospital A, reported details on case 10, admitted to the Paediatric ICU ward since December 4th 2013, with the diagnosis of pleural and lung infection with MRSA. The onset was 2 weeks before and the case was born in Hospital A Maternity on October 25th 2013. The case was considered as part of the same outbreak based on epidemiological criteria.

Cases 17 and 18 were admitted to the Paediatric Surgery ward, Hospital B on 26th and 29th of December, respectively, with skin abscesses caused by MRSA.

A parent call then announced the admission of his 14-day-old son to the Paediatric ward of Hospital D, sited in another city of the same county (case 19). The patient record mentioned the following diagnoses: staphylococcal skin infection, breast abscess, umbilicus granuloma.

Public health interventions

On December 20th 2013, a team of 4 sanitary inspectors evaluated the activity and hygiene conditions in Hospital A Maternity and applied a contraventional penalty for nonconformities found.

The epidemiologist from the Hospital Infection Control Service had delivered a minutes informing the Hospital management about pos-

sible shortcomings in newborns hygiene and the onset of several skin infections, but the Public Health Authority had not been asked for advice and methodological support.

Several nonconformities in respecting the refurbishing project were reported, as changing the destination of a room for nurses which was transformed into a room for washing and sterilisation of the instruments, thus causing unacceptable circuit breaks, and storage in the same room of euro pallets for waste collection. Remedies were taken.

Concomitantly, several other HCAI cases were detected, recorded in the patient observation sheets, but not reported. Thus, records of HCAI cases were belatedly drafted and included in the official reports of the Hospital for cases 8, 9, 12, 13, 14, 15, and 16.

Another finding of the inspection team was that many of the discharged newborns were mentioned with toxic or allergic erythema diagnosis on the discharge record: 59 cases in October, 43 cases in November, though the newborns were discharged very quickly, without being vaccinated for TB. The Chief neonatologist explained that these manifestations were normal and in his opinion did not represent a concerning situation.

After closing the ward, daily nebulization was done, with 3 alternative disinfectant types.

On December 22nd 2013, a first repeat of the microbiological environment control was performed, sampling a number of 38 air probes and 47 salubrity tests. All samples were negative, except 2 surfaces that proved positive for MRSA.

On December 23rd 2013, all HCWs underwent an evaluation test on cleaning, disinfection, and sterilisation.

All procedures of daily cleaning and disinfection were repeated and a new bacteriological control was performed on December 31st 2013. All samples proved negative.

On the same day, all 22 *S. aureus* carriers detected previously were sampled again, after a first round of therapy. Eight of them remained carriers and were advised to follow a new round of treatment. On January 24th 2014 no staff member was found to carry *S. aureus*.

On January 6th 2014 reopening of the Hospital A Maternity was decided based on accomplishing all recommended actions, negative environment controls and no reporting of other newborn infection cases.

During the outbreak investigation, 42 MRSA and MSSA strains were sent to the Iași Regional Public Health Centre, which confirmed MRSA by phenotypic methods (MRSA PBP2 latex test).

Microbiological investigation in the Reference Centre for Staphylococci/ Nosocomial Infections and Antibiotic Resistance Laboratory was done in the December 2013 - May 2014 interval.

Material and methods

Bacterial isolates

Fifteen *S. aureus* strains isolated from the HCAI first episode and 8 strains from 7 HCWs were received for confirmation and typing at the Reference Centre for Staphylococci/ Nosocomial Infections and Antibiotic Resistance Laboratory, "Cantacuzino" Institute. A single strain was selected per patient, but 2 patients presented 2 strains isolated from 2 different body sites (umbilical stump and skin). One HCW presented 2 different strains (1139-A and 1139-B) in the same biological probe (pharyngeal exudate) (Table 1).

From the second episode, six *S. aureus* strains were received for confirmation, typing and comparing with strains from the first episode (Table 1).

Table 1. Distribution of *S. aureus* strains by sampling site

Hospital samples	Number of isolates (isolate ID)
First episode	
Umbilical stump	4 (1122, 1124-A, 1127-A, 1128)
Nasal swabs (HCWs)	2 (1133, 1134)
Pustules	1 (1132)
Skin	7 (1120, 1124-B, 1125, 1127-B, 1129, 1130, 1131)
Abscess pus	2 (1121, 1123)
Lochia	1 (1126)
Pharyngeal exudates (HCWs)	6 (1135, 1136, 1137, 1138, 1139-A, 1139-B)
Second episode	
Abscess pus	2 (1305, 1306)
Baby changing radiant table in the birthing room	1 (1307)
Gynaecological examination table in the birthing room	1 (1308)
Nasal swabs (HCWs)	2 (1309, 1310)

Cultivation and characterization of the isolates

The isolates were cultivated on Columbia Blood Agar Base (Oxoid) with 7% sheep blood and Mannitol salt agar (Oxoid) and incubated at 35±2°C for 18-24h. Characteristic colonies were identified using standard phenotypic (including automated Vitek2, BioMerieux, France) and molecular microbiological methods. Triplex PCR for *nuc, lukS/F-PV* and *mecA* genes was performed using an optimized "*in house*" protocol (3).

Antibiotic susceptibility testing was performed by Kirby-Bauer disk diffusion method, according to the EUCAST standard v. 3.1 (4), and 4.0 (5) to a set of 15 antibiotics, using the *S. aureus* ATCC 29213 quality control reference strain. Vancomycin was tested by broth micro dilution method using the same quality control reference strain. The double-disk diffusion test for detecting inducible resistance to Macrolides, Lincosamides and Streptogramins B (D-test) was performed as described by Leclercq, 2002 (6).

Total genomic DNA from *S. aureus* strains used in all PCR reactions was obtained by thermal and enzymatic lysis method (lysostaphin 1U/ µL, proteinase K 2 mg/ mL).

Detection of *mec*A, *luk*S/F-PV genes was performed by the previously mentioned triplex PCR protocol and simplex PCR protocols were used for exfoliative toxins (*eta*, *etb*), toxic shock syndrome toxin 1 (*tst*1), benzylpenicillin (*blaZ* – encoding beta-lactamases responsible for resistance against penicillins), erythromycin-clindamycin [*erm*(A), *erm*(C) – encoding for methylases responsible for resistance against macrolides and clindamycin], gentamicin (*aac*A-*aph*D – encoding for a bifunctional aminoglycoside modifying enzyme), tetracycline [*tet*(K), *tet*(M) – responsible for tetracycline resistance] genes.

Sequences of primers used in triplex/ simplex PCR reactions and the cycling conditions were described in a previously published article (3). The PCR reactions were performed using an Applied Biosystems or a Corbett Research Thermocyclers.

The following reference strains were used as controls: 33/ 2009 strain (Nosocomial Infections Laboratory Collection) for *nuc*, *luk*S/F-PV, *mec*A genes; IC13456 strain (for *eta* gene); IC13455 strain (for *etb* gene); IC13454 strain (for *tst*1 gene); 5681/ 2010 strain (Nosocomial Infections Laboratory Collection) for *blaZ*, *erm*(C), *aac*A-*aph*D, *tet*(K) genes; 39/ 2008 strain

(Nosocomial Infections Laboratory Collection) for *erm*(A) and *tet*(M) genes.

The PCR products were migrated by agarose (1.5% and 2%) gel electrophoresis, stained with ethidium bromide 10 mg/ mL and visualized in an UV transilluminator.

SCCmec typing was performed by multiplex PCR according to the scheme proposed by Milheirico et al., 2007 (7). The following *S. aureus* reference strains were used as controls: COL (SCC*mec* type I), BK2464 (SCC*mec* type II), ANS46 (SCC*mec* type III), MW2 (SCC*mec* type IVa), WIS (SCC*mec* type V), HDE288 (SCC*mec* type VI).

Detection of polymorphism in the X region of the staphylococcal protein A (*spa*) gene (*spa*-typing) was carried out as described by Harmsen et al., 2003 (8) using the forward primer spa-1113f: 5'TAAAGACGATCCTTCGGTGAGC3' and the reverse primer spa-1514r: 5'CAGCAGTAGTGCCGTTTGCTT3'.

Finding of the *spa* repeat code and classification of strain into the *spa* types were done using the Ridom StaphType software v. 2.2.1 (Ridom GmbH, Wurzburg, Germany) and synchronized in the Ridom SpaServer (www.spaserver.ridom.de). The *spa* clonal complexes (*spa*-CC) were deduced using the Based Upon Repeat Pattern algorithm (BURP).

Pulse Field Gel Electrophoresis (PFGE) was performed using the "gold standard" method previously described by Murchan et al., 2003 (9). *S. aureus* NCTC 8325 was used as size marker to allow later normalization of electrophoretic patterns across the gel. The digital image was stored electronically as TIFF file and analyzed with the BioNumerics 6.6 (Applied Maths) software using the Dice coefficient and UPGMA method with 1.5% tolerance and 1.5% optimization settings. A similarity cut-off of 80% (2 different bands) and the criterion of a difference of no more than 6 bands as described by Tenover et al., 1995 were used to define a pulsotype (10).

Results

All the strains proved *nuc* gene positive, except one (1310 strain) identified as *Staphylococcus haemolyticus* using the Vitek2 system. The origin of strains by sample and their PFGE, *spa*, SCC*mec* types and the presence of *luk*S/ F – PV genes are presented in Figure 1.

All the strains proved negative for the exfoliative toxins A and B (*eta*, *etb*), the toxic shock syndrome toxin 1 (*tst*1), aminoglycosides (*aac*A-*aph*D), and tetracycline [*tet*(M)] genes. The 1124-B strain was positive for *erm*(C) gene, and the 1133 strain was *erm*(A) gene positive. At a similarity index of 80% between the band profiles obtained in the PFGE analysis ten pulsotypes designed A-J and three subtypes (D1 and D2; E1 and E2; G1 and G2) were distinguished.

Seven strains isolated from 6 newborns and a HCW nasal swab from the first episode and four strains isolated from abscess pus (2 strains), baby changing radiant table and gynaecological examination table in the birthing room from the second episode classified as PFGE pulsotype A (100% similarity index, 0 difference bands which demonstrates their clonality), with the same antibiotic resistance pattern P, FOX, E, K, *spa* type t008, SCC*mec* IV type, and *bla*Z, *mec*A, *luk*S/F-PV genes positive; one strain isolated from the labour associated infection (lochia) and two strains from the skin of two newborns classified as PFGE pulsotype D subtype D1 (100% similarity index, 0 difference bands which demonstrates their clonality), *spa* type t015, *bla*Z gene positive, resistant to P; two strains isolated from newborn umbilical stumps from the first episode classified as PFGE pulsotype G, subtype G1 (100% similarity index, 0 difference bands which demonstrates their clonality) and one strain from a HCW nasal swab from the second episode classified as PFGE pulsotype G, subtype G2 (86% similarity index with subtype G1, 3 difference bands which proves they are close-

ly related), with antibiotic resistance pattern P, FOX, K, *spa* type t002, SCC*mec* IV type and *bla*Z, *mec*A genes positive; one strain from the pharyngeal exudate of a HCW and two strains from newborns' skin classified as PFGE pulsotype E, subtypes E1 or E2 (91% similarity index, 2 difference bands which proves they are closely related), antibiotic resistance pattern P, FOX, E, DA (MLSBi), K, TE/ TE(I), QD, *spa* type t127, SCC*mec* IV or IVE types and *bla*Z, *mec*A, *erm*(C) and *tet*(K) genes positive. A strain belonging to this cluster was positive for *luk*S/F-PV genes. PFGE clusters dendrogram of the *S. aureus* isolates is presented in Figure 1.

Discussion

Staphylococcus aureus is a bacterium that commonly colonizes the skin and mucosal surfaces of many mammalian and avian species, but it may become a challenging and costly cause of skin and soft-tissue infections (SSTIs), endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign-body infections, sepsis, especially in immunocompromised patients and toxinoses, most frequently food poisoning with preformed Staphylococcal Enterotoxins (SEs). The nasal cavity is the most epidemiologically important site for colonization in humans, and estimates of prevalence of permanent nasal carriage of *S. aureus* by people are of 20% to 30% (11).

Under antibiotic pressure, but also as a measure of adaptation to various environmental conditions, *S. aureus* has changed its chromosomal structure by mutation or horizontal gene transfer and has become multidrug resistant and virulent.

In a healthcare facility, the sources of infection and of the preceding contamination may be the staff, the infected or colonized patients, household members and other visitors, or the in-

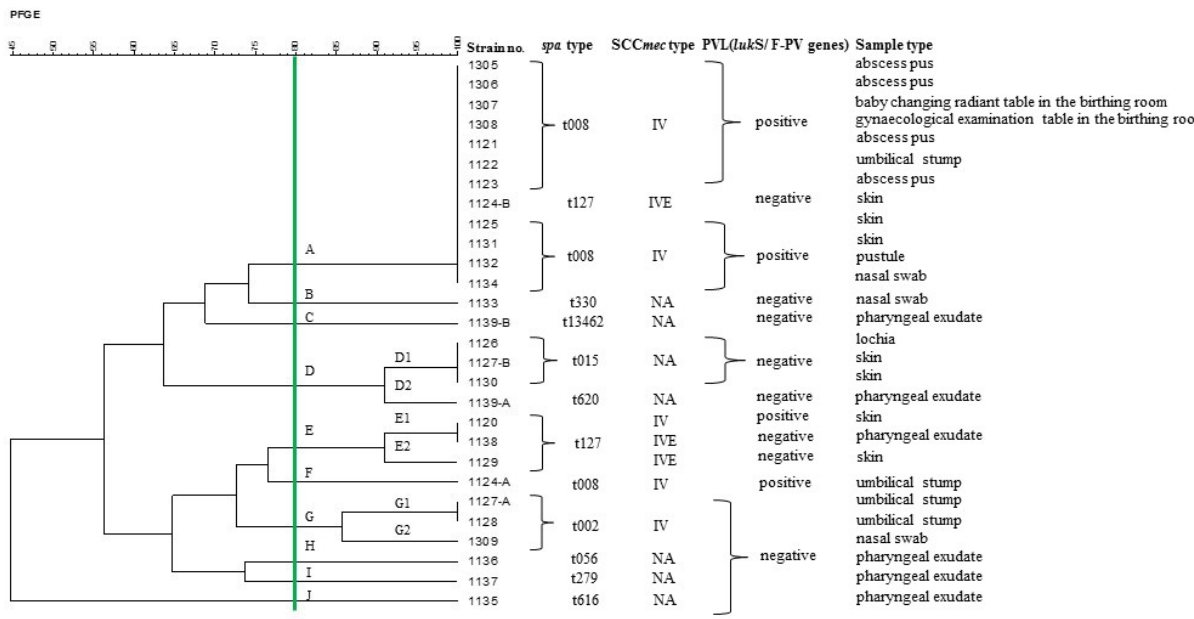


Fig. 1. PFGE clusters dendrogram of the *S. aureus* isolates from the first and second episodes analyzed using BioNumerics software v. 6.6 (Applied Maths). The predominant strains of the outbreak were framed in the PFGE pulsotype A (100% similarity index, 0 difference bands) with *spa* type t008, positive for *luk*S/F-PV genes.

animate environment. The hospital environment may be contaminated with multidrug resistant or virulent pathogens. Personal protective equipment (PPE) may become contaminated with potential pathogens after care of a patient colonized or infected with an infectious agent (12).

According to Albrich and Harbarth, transmission of MRSA from staff to patients was demonstrated by genotyping in 63 of 68 studies (93%) and MRSA eradication was successful in 449 of 510 healthcare staff (88%) (13).

During general care and/ or medical treatment, the hands of HCWs often come in close contact with patients. The hands of the clinical staff are thus the most frequent vehicles for healthcare-associated infections, if hand hygiene is not performed before touching another patient. Transmission by this route is much more common than vector-borne or airborne transmission or other forms of direct or indirect contact. Rammelkamp et al. demonstrated that infants exposed to nurses who handled colonized newborns acquired *S. aureus* in 14% of cases and 43% of cases if good, respectively no handwashing was performed (14). The good compliance with hand hygiene is essential to prevent cross-transmission.

In his PhD thesis, Mernelius, demonstrated that transmission of *S. aureus* through air is not important, because no *S. aureus* was detected in the air (15).

However, Sherertz et al. recently supported the evidence of *S. aureus* outbreaks associated with skin colonization or viral upper respiratory tract infection by a phenomenon of airborne dispersal called the "cloud" phenomenon. Though airborne dispersal was insignificant with nasal breathing, counting, coughing 6 times, or sneezing once, moving produced large numbers of *S. aureus* in the air, most probablly dispersed from skin and clothing by friction and movement (16).

On the other hand, all objects that come in contact with patients should be considered potentially contaminated. Indirect contact transmission may include: patient-care devices (e.g., electronic thermometers, glucose monitoring devices) which may transmit pathogens if devices contaminated with blood or body fluids are shared between patients without cleaning and disinfecting between uses; shared toys may become a vehicle for transmitting pathogenic bacteria among paediatric patients; instruments that are inadequately cleaned between patients before disinfection or sterilization (e.g., endoscopes or surgical instruments) or that have manufacturing defects that interfere with the effectiveness of reprocessing may transmit bacterial pathogens (17).

The first essential measure in preventing the spread of healthcare-associated infections with MRSA is isolation of infected patients in order to reduce the spreading of pathogens.

Unit-based procedures, e.g. visitation, hand washing and nail care, skin and cord care, adequate maintenance of hubs in peripheral and central lines, correct cohorting and isolation procedures, appropriate use of antibiotics, and adequate unit design and staffing are recommended in order to minimize infectious risk in NICUs (1).

In 2013, Chan et al. found evidence supporting overall higher levels of early-onset neonatal infection in the newborns of mothers with bacterial infection or colonization compared to the newborns of mothers without infection or colonization, with seven times higher odds of early-onset neonatal infection in newborns of infected mothers compared to newborns of uninfected mothers (18).

In the present reported outbreak there is an unusually high rate of infant breast infections. Though usually uncommon, breast infections may be favoured by local hormonal conditions that may be present in this period of life, mostly in the second week of life, when neonatal breast tissue is still enlarged because of the maternal oestrogens (19).

Until recently, MRSA infections presenting in the community were usually caused by healthcare-associated MRSA (HCA-MRSA) strains acquired directly or indirectly during previous hospital or healthcare contact (20).

Starting with the late 1990s, Community-Associated MRSA (CA-MRSA) strains were introduced from the community into hospitals, causing hospital-associated infections and outbreaks (21). Community based infection control strategies may still be helpful in stemming the influx of traditionally community-associated strains, particularly USA300, into the healthcare setting.

USA300 (t008/ ST8/ SCC*mec*IV) is the predominant, traditional CA-MRSA clone in the US that has rapidly disseminated and replaced USA400 (ST1 and *spa* types t127, t128 and t1178) since its isolation in 2000 and then spread worldwide (22).

The success MRSA clone USA300, susceptible to clindamycin, trimethoprim/ sulfamethoxazole and gentamicin, evolved initially in semi-closed collectivities and started to spread in hospital units in 2000 (23). The first infection caused by MRSA USA300 evolving in a NICU was reported in 2004 (24). This strain was involved in SSTIs and severe invasive infections like necrotizing pneumonia, osteomyelitis, septic arthritis, etc., because of its capacity to produce several virulence factors like Panton-Valentine leukocidin (PVL), exfoliative toxins etc. (22).

In infection control and healthcare epidemiology, the clinical microbiology laboratory plays a critical role, contributing to the prevention of infectious disease transmission in healthcare settings by promptly detecting and reporting epidemiologically important organisms, identifying emerging patterns of antimicrobial resistance, and assisting in the assessment of the effectiveness of recommended precautions to limit transmission during outbreaks (25). Laboratories are usually the first to recognize infection outbreaks.

The introduction of molecular typing methods, including Whole-Genome Sequencing (WGS) into epidemiological investigations has provided powerful new tools for tracking the origin and routes of dissemination of MRSA strains not only among patients in a hospital, but also among hospitals and community, in different countries or even across different continents (26). The "gold standard" to track bacterial transmission in healthcare settings has for long time been considered to be the Pulsed Field Gel Electrophoresis (PFGE), a method that is labour-intensive and relies on semi-subjective analysis. More discriminatory, Multi Locus Sequence Typing (MLST) is laborious and time-consuming, while molecular typing by sequencing of the hyper variable part of the *S. aureus* protein A gene (*spa* typing) has overcome these problems and has shown promising results in epidemiological investigations (15). Typing of Staphylococcal Chromosomal Cassette *mec* (SCC*mec* typing) may provide additional information on *S. aureus* geographical and temporal evolution.

The most disseminated HA-MRSA worldwide includes the ST239 lineage (27, 28), as well as the ST5 lineage (29). The most characterized CA-MRSA includes the ST8 lineage, such as ST8/ SCC*mec*IVa (USA300) (30), and also the lineages of ST30/ SCC*mec*IV, ST80/ SCC*mec*IV (31), and ST59/ SCC*mec*V (32). CA-MRSA mainly causes SSTIs, but also, occasionally, invasive infections, and often produces PVL (31) and exhibits elevated expression of cytolytic peptides (phenol-soluble modulins, PSMs; or δ-hemolysin, Hld) (33).

CA-MRSA bacteria are much less prevalent in Europe, where the European ST80-IV CA-MRSA clone, USA300 CA-MRSA strains, and other lineages, such as ST22-IV, coexist.
Infections with PVL-positive MRSA have been described in most European countries as Austria (34), Germany (35), Bulgaria (36), Spain

(37), UK (38), Italy/ NICU (39), Belgium (40), France (41).

Romania is considered a "hot spot" for MRSA infections (42).

According to the report of the European Antibiotic Surveillance Network (EARS-Net) in Romania, the percentages of MRSA in invasive infections were 49.5% in 2011, 53.3% in 2012, 64.5% in 2013 and 56% in 2014 (http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-europe-2014.pdf).

Case reports on fatal sepsis due to PVL-positive CA-MRSA *spa* type t044 clone were presented in one study (43), and one communication (44) by Romanian authors. Over the time, PVL-positive t044 *S. aureus* strains have been involved in necrotizing pneumonia, fatal sepsis with community-onset, associated with influenza, but also with skin infections evolving in teenagers or pre-school children in Romania (43, 44).

Reported MRSA rates in hospitals are very high, ranging from approximately 30% up to 85%, whereas CA-MRSA has been identified inside hospitals (45-56). *S. aureus* s*pa* type t030 has been described as the prevalent MRSA *spa* type in Romania (57), being reported in several studies as the most widespread MRSA in Romanian hospitals (50, 58), and considered as being endemic (59). Second Seqnet.org structured survey (60) and other authors (61) reported the ST239 clone as the prevalent one. CA - *S. aureus spa* type t008 was reported in 2008, in one Romanian western county hospital (62). *S. aureus* s*pa* type t127 has been reported frequently in Romanian hospitals starting with 2006 (58, 59, 63). This *spa* type was described also by Franco & al., in 2011 to be associated with animals (e.g., pigs) (64). Recently, three PVL-negative MRSA t127 strains, with the same PFGE pulsotype were isolated from newborn skin, from the bed frame and hospital air controls in one Bucharest hospital (unpublished data).

Monecke et al. published a study on clinical isolates recovered between 2008-2012 from bloodstream infections, SSTIs, as well as from screening swabs collected from several hospitals in Iași, the North-Eastern part of Romania. Isolates were characterised by microarray hybridisation and nearly half of all isolates (47%), and about one third (34%) of bloodstream isolates were MRSA. Thirty one percent (31%) MRSA and 14% MSSA strains were positive for PVL genes (65).

In 2014, Scheithauer et al. published a report on a MRSA *spa* type t127 outbreak evolving a neonatology level III ICU. This *spa* type, extremely rare in Germany, proved to come from a HCW belonging to a group of recently employed Romanian HCWs, who was colonized with the *spa* t127 strain. All carriers were successfully decolonized. After this outbreak, a universal screening programme was implemented in the hospital, of all new employees, as part of infection control management strategy (66).

Molecular analysis of strains isolated in the present reported outbreak revealed a strong circulation of several *S. aureus* clones, some producing Panton-Valentine leukocidin, and cross-contamination of newborns, HCWs, a mother with a labour associated infection and the inanimate environment. Genotyping of *S. aureus* strains made possible the tracking of the sources and routes of transmission and evaluating the potential of pathogenicity and substantiated the public health interventions.

The predominant clone of the outbreak was MRSA t008/ PVL-positive/ PFGE pulsotype A/ P, FOX, E, K/ *bla*Z and *mec*A-positives/ SCC*mec*IV, followed by MSSA t015/ PVL-negative/ PFGE pulsotype D subtype D1/ P/ *bla*Z-positive and *mec*A-negative; MRSA t127/ PVL-negative or positive/ PFGE pulsotype E subtypes E1 or E2/ P, FOX, E, DA (MLSBi), K, TE or TE(I), QD/ *bla*Z, *mec*A, *erm*(C), *tet*(K)-positives/ SCC*mec*IV or IVE; MRSA t002/ PVL-negative/ PFGE

pulsotype G subtypes G1 or G2/ P, FOX, K/ *bla*Z and *mec*A-positives/ SCC*mec*IV.

Among the different interventions used to stop outbreak spreading, the most fruitful reported ones have been the implementation of improved hand hygiene, gloves and gowns for patient care, patient and staff decolonization (mupirocin intranasal ointment, bathing with antiseptics as povidone iodine, clorhexidine or hexachlorophene), isolation of colonized patients, cohorting of patients and nurses, staff training, etc. (67).

Laboratory based interventions like surveillance cultures for patients, staff and environment, molecular typing of strains, etc. are considered the most useful for identifying sources and ways of transmission and evaluating the intervention impact (68).

As there are no unanimously accepted guidelines, Gerber et al. published a consensus statement in 2006 on the prevention and control of MRSA colonization and infection, including, besides the aforementioned interventions starting with hand hygiene, cohorting and isolation, open communication between NICUs, collaboration with public health institutes, keeping strictly close to appropriate staffing and avoiding crowding of the newborn units (69).

Conclusion

We considered reporting this HCAI prolonged episode originating in a newborn unit, taking into account the unusual evolution, the risk of multiresistant bacterial strain spreading, and multiple unwanted consequences caused by shortcomings in providing appropriate hygiene conditions.

Though the outcome was favourable in all cases, observing hygiene regulations and avoiding shortcomings, earlier recognition of the outbreak using laboratory findings, along with prompt communication with public health institutions would result in fewer cases, with less financial expenses, and unwanted medical complications.

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This publication made use of the *spa* typing website (http://www.spaserver.ridom.de/) that is developed by Ridom GmbH and curated by SeqNet.org (http://www.SeqNet.org/).

Results of this work were presented partially at the National Conference of Microbiology and Epidemiology, November 2014, Bucharest, Romania and are part of the doctoral thesis of E.C.D.

Authors' contribution

E.C.D. – study design and microbiological investigation, data analysis and interpretation, wrote the manuscript with support by I.C.
M.O. – data analysis and interpretation
C.Z. – field outbreak investigation and epidemiological report
R.S. – final epidemiological report and integration of microbiological information
I.C. – study design, integration of epidemiolog-

ical data, contributed to the final version of the manuscript, final approval of the version to be published.

Conflict of interest

The authors declare that they have no conflict of interests.

Abbreviations

Staphylococcus aureus: *S. aureus*
Healthcare workers: HCWs
World Health Organization: WHO
Healthcare-Associated Infection: HCAI
Newborn Intensive Care Units: NICUs
Tuberculosis: TB
Methicillin-Resistant *S. aureus*: MRSA
Intensive Care Unit ward: ICU ward
Methicillin-Susceptible *S. aureus*: MSSA

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