



Epidemiological typing of methicillin resistant *Staphylococcus aureus* recovered from patients attending a maternity hospital in Ireland 2014–2019

Deirdre Broderick^{a,*}, Gráinne I. Brennan^b, Richard J. Drew^{c,d,e}, Brian O'Connell^{a,b}

^a Department of Clinical Microbiology, St. James's Hospital, James's St., Dublin 8, Ireland

^b National MRSA Reference Laboratory, St. James's Hospital, James's St., Dublin 8, Ireland

^c Clinical Innovation Unit, Rotunda Hospital, Parnell Square, Dublin 1, Ireland

^d Irish Meningitis and Sepsis Reference Laboratory, Children's Health Ireland at Temple Street, Dublin 7, Ireland

^e Department of Clinical Microbiology, Royal College of Surgeons in Ireland, Dublin 2, Ireland

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SUMMARY

It has previously been reported that maternity hospitals have lower levels of MRSA compared to other healthcare facilities, due to the patient population - mostly healthy patients with limited healthcare contact. In this epidemiological study, all MRSA isolates recovered from patients attending a maternity hospital from 2014 – 2019 were investigated. 171 isolates from adults (n=120) and babies (n=51) from diagnostic and screening investigations were submitted to the National MRSA Reference Laboratory (NMRSARL). Investigations included: *spa* typing, antimicrobial susceptibility testing, detection of the *mecA/mecC* genes and *lukS-PV* and *lukF-PV*. All were susceptible to glycopeptides, linezolid, rifampicin and mupirocin, while 29 of 171 (17%) were resistant to β -lactam agents only. Thirteen isolates (8%) were resistant to two classes of antibiotic; one resistant to three. All isolates harboured *mecA* and 33 of 171 (19%) harboured *PV-lukF/S*. Among the collection, 21 multilocus sequence types (ST) were inferred from 63 *spa* types. EARS-NET data shows that ST22-MRSA-IV accounts for approximately 75% of MRSA recovered in Irish hospitals. Here, it accounted for only 25.7%. MLST types associated with community acquired MRSA accounted for the remaining 74.3%. These included ST8, ST30, ST1, ST5 and ST88, suggesting a diverse population, harbouring multiple resistance and virulence genes, some of which have been previously associated with outbreaks in Ireland. This study exposes a reservoir of MRSA in the community which may be imported into hospitals, leading to outbreaks. The diversity of MRSA lineages with enhanced virulence factors highlights the need for regular surveillance to ensure appropriate infection prevention and control interventions are implemented promptly.

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* Corresponding author. Address: Department of Clinical Microbiology, St. James's Hospital, James's St., Dublin 8, Ireland.

E-mail address: broderdm@tcd.ie (D. Broderick).

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen frequently associated with serious and often life-threatening infections in individuals with established risk factors, such as prolonged hospital stay and antibiotic usage, older age or recent surgery [1]. These healthcare-associated MRSA (HA-MRSA) have been found to be limited to five predominant lineages with worldwide distribution [2].

Since the late 1980s a distinct change in the epidemiology of MRSA has been described with the emergence of community-associated MRSA (CA-MRSA). Initially these CA-MRSA strains were reported among individuals living in remote communities in Western Australia and were associated with the Panton-Valentine leukocidin (PVL) toxin [3]. These strains have also been shown to be genotypically distinct to HA-MRSA. Moreover, they exhibit greater diversity with increased resistance and virulence genes having different lineages associated with specific geographical regions [1,4,5]. More recently however, with increasing reports of PVL detection among MRSA recovered in healthcare facilities and MSSA isolates, PVL is no longer considered a suitable indicator for community associated infection [5].

Within healthcare settings, several studies have reported the displacement of previously predominant HA-MRSA clones by CA-MRSA clones. This has been observed in India, where the multidrug resistant CA PVL-positive CC1-ST772-MRSA-V clone is displacing the previously predominant HA ST239-MRSA-III clone [6]. Similarly, in the USA, the CC8-ST8-MRSA-IV clone, also known as USA300, now accounts for the majority of MRSA nosocomial infections, having displaced the HA CC5-ST5-MRSA-II clone, USA100 [5]. In Ireland, CA-MRSA strains have also been linked to outbreaks in hospitals with several having occurred in neonatal intensive care units (NICU) [7,8]. These strains have also frequently been associated with travel and in particular to areas where these CA-MRSA strains are endemic [8].

Similar to elsewhere in Europe, ST22-MRSA-IV has been the predominant strain in Irish hospitals for the past two decades, accounting for approximately 80% of isolates recovered from MRSA bloodstream infections (BSI). In contrast, isolates recovered from sites other than BSI are more diverse with the predominant strains including ST8, ST5 and ST1 [9].

It has previously been reported that maternity hospitals often have lower levels of MRSA colonisation in comparison to other healthcare facilities. In the USA, a study from 2009 showed an anogenital carriage rate of MRSA of 0.5% in pregnant women, a rate that had remained stable since 2005 when it was last assessed [10]. A similar rate was seen in another U.S. study in 2006 [11]. In Europe, a Scandinavian study from 2008 found a colonisation rate of 0.7% in neonates and 2.1% in mothers [12]. However, no recent prevalence studies have been conducted. This has been attributed to the select patient population utilising these facilities [13]. Despite this, it has also been suggested that the frequency with which MRSA has caused infection among pregnant women and neonates has increased [14]. In particular, horizontal transmission of MRSA from colonised mothers may play a role in an increased level of neonatal colonisation [15]. Following a three year study in an Irish maternity hospital, the prevalence of MRSA carriage in

pregnant women in Ireland was found to be 1.6% (unpublished data) while a large study in the U.K. found 0.5% of pregnant women were nasal carriers of MRSA [16].

It has been shown that the prevalence of CA-MRSA in some areas may range from 0-23.5% [17]. However, to date no CA-MRSA prevalence studies have been carried out in Ireland resulting in a lack of information about CA-MRSA and potential reservoirs of CA-MRSA in the community. The aim of this project was to investigate the epidemiology of MRSA isolates recovered from patients attending a large maternity hospital, many of whom have no previous healthcare exposure risks and represent possible cases of CA-MRSA. The study aim was to examine these isolates submitted over a six year period in order to determine their clonal relatedness, virulence and resistance profiles.

Setting

This project was undertaken in the Rotunda Hospital, a tertiary referral standalone maternity hospital founded in 1745. The hospital is the oldest continuously functioning maternity hospital in the world (consisting of 188 beds), which includes a neonatal intensive care unit. Approximately 8,500 babies are delivered each year. [18].

Methods

Isolates were recovered during routine microbiological investigations and were submitted to the National Methicillin Resistant *Staphylococcus aureus* Reference Laboratory (NMRSARL) for investigation. Duplicate and staff samples were removed. Isolates were recovered from screening samples (69%, 118/171), which were collected during routine screening procedures within the hospital (including weekly in the neonatal intensive care unit and voluntary preadmission screening of healthcare workers) and from diagnostic samples (31%, 53/171). All isolates underwent phenotypic susceptibility testing against a panel of antimicrobial agents including gentamicin, amikacin, kanamycin, neomycin, streptomycin, tobramycin, erythromycin, lincomycin, linezolid, vancomycin, fusidic acid, tetracycline, ciprofloxacin, rifampicin, trimethoprim and mupirocin using disk diffusion as previously described [19]. Isolates were also investigated for the presence of the PVL encoding genes (*PV-lukF/S*), confirmed to harbour the *mecA/mecC* genes [20] and underwent *spa* typing to determine the relatedness of isolates recovered in this institution [21]. Where possible, the MLST was inferred from the *spa* type to enable comparison with international lineages. The clinical details of each patient from whom the isolate was recovered were also reviewed to determine the patients' ethnicity and the isolate source. Approval was granted by the Research Advisory Group (a subcommittee of the Research Ethics Committee, reference code RAG-2020-004) in the Rotunda for this data to be collected.

Results

Bacterial isolates

Between 2014 and 2019, 171 MRSA isolates were recovered from babies (n=51) and adult patients (n=120) during routine

Table 1Epidemiological typing of methicillin resistant *Staphylococcus aureus* recovered from patients in a maternity hospital between 2014 and 2019

Inferred MLST ^a	Patient ethnicity (n)	spa type (n)	PVL(n)	Antimicrobial profile ^b (n)
ST22 (44)	Irish (16), Other (7), Not known (21)	t005 (2), t022 (3), t032 (22), t223 (3), t309, t608, t756, t891, t1205, t2945 (3), t3799, t4422, t13735, t13454, t14339, t17846	Positive (4) Negative (40)	Gn (3), Kn (3), Str (1), Ery (15), Ln (1), Fd (21), Te (2), Cp (36), Tp (10)
ST8 (38)	Irish (13), Other (16), Not known (9)	t008 (18), t064 (2), t304 (12), t1610 (2), t723, t1476, t1578, t4146	Positive (18) Negative (20)	Gn (2), Ak (3), Kn (15), Nm (14), Tb (3), Ery (20), Ln (1), Fd (2), Te (6), Cp (19), Tp (3)
ST1 (26)	Irish (8), Other (12), Not known (6)	t127 (21), t1784, t2279 (2), t3636, t948	Positive (1) Negative (25)	Gn (4), Ak (1), Kn (23), Nm (21), Str (18), Tb (4), Ery (23), Fd (8), Te (17), Cp (7)
ST5 (25)	Irish (6), Other (14), Not known (5)	t002 (6), t010 (5), t111 (1) t242 (8), t311 (2), t3632, t688, t2516	Negative (25)	Kn (1), Nm (1), Tb (1), Ery (11), Ln (1), Fd (7), Te (3), Cp (11), Tp (4)
ST88 (8)	Irish (4), Not known (2), Other (2)	t690, t786 (3), t878, t1339 (2), t2177	Positive (2) Negative (6)	Ery (3), Fd (1), Te (3), Tp (1)
ST30 (6)	Other (3), Not known (2), Irish (1)	t012 (2), t019 (3), t127	Positive (5) Negative (1)	Gn (1), Kn(2),Nm (2), Tb (1), Ery(2), Fd (2), Cp(2), Tp (1)
ST672 (3)	Other (3)	t3841 (2), t17271	Negative (3)	Gn (1), Hn (1), Tb (1), Fd (1), Cp (3)Tp (3)
ST7 (3)	Irish (1), Not known (2)	t091	Negative	Gn, Ak, Kn, Nm, Tb, Te, Cp, Tp
ST6 (2)	Irish (1), Other (1)	t3967, t18492	Negative	Fully susceptible
ST772 (2)	Other (2)	t657 (2)	Positive (2)	Gn (2), Ak (1), Kn (2), Nm (2), Tb (2), Ery (2), Cp (2), Tp (2)
ST487 (2)	Other	t442	Negative	Gn (1), Kn (1), Tb (1), Ery, Cp, Tp
ST97 (2)	Other (1), Irish (1)	t359, t7433	Negative	Fully susceptible
ST45 (2)	Other (1), Irish (1)	t230, t728	Negative (2)	Fully susceptible
ST2250 (1)	Not known	t6675	Negative	Fully susceptible
ST1430 (1)	Other	t451	Negative	Tet, Cp, Tp
ST39 (1)	Irish	t007	Negative	Gn, Kn, Nm, Tb, Ery, Ln
ST46 (1)	Other	t132	Negative	Ery
ST59 (1)	Not known	t976	Negative	Ery
ST79 (1)	Irish	t779	Positive	Fully susceptible
ST779 (1)	Irish	t878	Positive	Fully susceptible
ST152 (1)	Asian	t4690	Negative	Tet, Tp

(n) Number of isolates associated with each characteristic.

^a Multilocus Sequence Type (MLST) was inferred based on the *spa* type assigned to the isolates using Ridom StaphType software (Ridom GmbH, Würzburg, Germany) with an online database available at <https://www.spaserver.ridom.de/>.^b Antimicrobial resistance was determined by antibiogram-resistogram typing against a panel of 23 antimicrobial agents including Ak, amikacin; Gn, gentamicin; Kn, kanamycin; Nm, neomycin; Str, streptomycin; Tb, tobramycin; Amp, ampicillin; Ery, erythromycin; Fd, fusidic acid; Te, tetracycline; Van, vancomycin; Rf, rifampicin; Cp, ciprofloxacin; Mp, mupirocin; Tp, trimethoprim; Ln, linezolid; Fx, ceftioxin; Su, sulphonamides; CdAc, cadmium acetate; PMA, phenyl mercuric acetate; Sp, spectinomycin; Ln, lincomycin.

microbiology diagnostic investigations. Among the collection, 17 isolates (9.9%) were recovered in 2014, 13 (7.6%) in 2015, 18 (10.5%) in 2016, 39 (22.8%) in 2017, 48 (28.1%) in 2018 and 36 (21.1%) in 2019.

Whilst the majority of isolates (69%, 118/171) were recovered during routine screening procedures, the remaining 31% (53/171) of samples were collected due to concern regarding infection. The sample sites included abdominal swabs (22.6%, 12/53) and wound swabs (20.7%, 11/53). The wound sites were unspecified. The remaining samples (56.6%, 30/53) were collected from multiple sites including high vaginal swabs, breast milk, urine, swabs of the umbilicus and skin, an eye swab and a blood culture. Patients attending outpatient clinics accounted for 53.8% (92/171) of isolates with 42.7% (73/171) recovered from inpatients. No location was recorded for 3.5% (6/171) of the patients from whom samples were taken.

While the ethnic origin of 28.7% (49/171) of patients was unknown, 31.6% (54/171) were Irish. Other ethnicities among the patient population included Asian, African, Roma, mixed ethnicities, and European (Table I).

Antibiotic susceptibility

All isolates were confirmed as MRSA through the presence of *mecA* and exhibited phenotypic resistance to β -lactams agents. All isolates were susceptible to glycopeptides, linezolid and rifampicin.

Among the collection, 17% (29/171) were resistant to only the β -lactam agents, 34% (52/171) exhibited resistance to at least one aminoglycoside, 10.5% (18/171) to erythromycin, 50.3% (86/171) to ciprofloxacin, 21.1% (36/171) to tetracycline, 25.2% (43/171) to fusidic acid and 18.1% (31/171) to trimethoprim. Two isolates (1.2%, 2/171) were resistant to mupirocin. Thirteen isolates (8%) were resistant to two classes of antibiotic while only one isolate was resistant to three classes (Table I).

Epidemiological typing

spa typing identified 63 different *spa* types with the commonest being t127, t008, t032 and t242. The inferred ST included ST22 (25.7%, 44/171) and ST8 (22.2%, 38/171). *spa* types were also associated with ST1 (15.2%, 26/171), ST5 (14.6%, 25/171), ST30 (3.5%, 6/171), ST88 (2.34.7%, 8/171), ST7 (1.8%, 3/171) and ST672 (1.8%, 3/171). ST772, ST6, ST97, ST487 and ST45 each accounted for 1.7% (2/171). The remaining sequence types recognised were each associated with 0.6% (1/171) each (Table I).

Nineteen per cent of isolates (33/171) were found to harbour *PV-lukF/S* which encodes the PVL toxin. Within the predominant sequence types, the majority of the isolates designated ST30 were found to harbour the PVL genes (83.3%, 5/6), while all those assigned to ST5 lacked these genes. Other sequence types including ST1 (3.8%, 1/26), ST8 (44.7%, 21/47), ST772 (100%, 2/2), ST22 (9.1%, 4/44) and ST88 (25%, 2/8) included PVL positive and PVL negative isolates (Table I).

Forty-four isolates were assigned to ST22, with *spa* type t032 which accounted for 50% (22/44) of isolates. Of these samples, the majority (54.5%, 24/44) were recovered from outpatients; two had no location stated while the remaining 45.5% (20/44) were recovered from inpatients. The ethnicity of patients from whom ST22 isolates were recovered was not recorded for 47.7%

(21/44) of patients. Ethnicity was recorded as Irish in 36.3% of patients (16/44) and 'other' in 15.9% (7/44). A majority (84.1%, 37/44) were resistant to ciprofloxacin with twenty-one (47.7%, 21/44) displaying resistance to fusidic acid and fifteen (34.1%, 15/44) being resistant to erythromycin.

Although 38 isolates were assigned to ST8, 47.4% (18/38) of these were *spa* type t008 with the remaining isolates being assigned to seven other *spa* types. Thirteen isolates were recovered from Irish patients while a further 16 had epidemiological links outside of Ireland. During the period of the study, ST8 MRSA was recovered from 21 patients (21/38, 55.3%) who were attending outpatient clinics or the Emergency Dept. at the time of sample collection. Seven isolates (18.4%, 7/38) were recovered from clinical samples due to concern regarding infection, and the remainder were isolated from screening samples. Antimicrobial resistance varied among isolates with ciprofloxacin resistance being most frequently detected in 50% (19/38) of isolates. The PVL genes were detected in 47.4% (18/38) of isolates (Table I).

The ST1 group contained five *spa* types [t127 (21), t1784 (1), t2279 (2), t3636 (1), t948 (1)]; twenty three of which exhibited resistance to macrolides (88.5% with 65.4% (17/26) demonstrating resistance to tetracyclines. At the time of sample the collection, 23.1% (6/26) of patients were infants in the NICU, with the remaining isolates recovered from adult patients, the majority of which were outpatients. (75%; 15/20).

The predominant *spa* type among ST5 isolates was t242 (32%, 8/25), with t002 and t010 each accounting for 24% (6/25) and 20% (5/25) respectively (Table I). Ciprofloxacin and erythromycin resistance was recognised in 44% (11/25) of isolates. Among ST5, 36% (9/25) of the patients were babies in the NICU at the time of sampling, with the remainder of isolates recovered from adult inpatients (24%, 6/25) or outpatients. (24%, 6/25).

Within the remaining 38 isolates, 17 separate MLST groups were recognised (Table I), with a range of one to eight isolates per MLST. Of the 22.2% (38/171) of isolates that did not belong to the four main MLST (ST1, ST5, ST8, ST22) 26.3% (10/38) were positive for PVL, with the remainder testing negative.

Discussion

It has previously been reported that the epidemiology of MRSA in Ireland is changing [1,4,8,9,22,23] and this study has highlighted a diverse population of MRSA recovered from patients in a maternity hospital over a prolonged period of time which included 63 *spa* types associated with 21 ST. The major lineages recognised include ST22, ST8, ST1 and ST5, each of which have been previously associated with CA-MRSA lineages and of which ST1, ST772 and ST88 have also been associated with outbreaks in Irish healthcare facilities [7,8,23].

Frequently, MRSA in Irish healthcare facilities is caused by ST22-MRSA-IV accounting for approximately 75% of MRSA recovered from blood stream infections [9]. In contrast, here we have shown that ST22-MRSA accounted for only 25.7% (44/171) of the population. This may be reflective of the patient population attending the hospital where many have had no previous exposure to other healthcare facilities where ST22-MRSA predominates, and also the high rate of non-Irish patients attending the service. The ST22-MRSA-IV strain often exhibits resistance to β -lactam antibiotics, fusidic acid,

ciprofloxacin, and erythromycin and this susceptibility pattern was reflected in the isolates examined in this study. In addition while HA-ST22-MRSA-IV lacks many resistance and virulence genes [22] but distinct to HA-ST22-MRSA-IV, a CA-ST22-MRSA lineage harbouring *pvl* has emerged and been reported in several countries including Ireland and India [1,6]. In the current study, three ST22-MRSA isolates were found to harbour the PVL genes and, while two of these isolates were recovered from screening samples collected from infants admitted to the NICU, one was recovered from an adult attending an outpatient clinic. Whilst there was no known epidemiological links among these three patients to countries outside of Ireland, differences between the susceptibility profile of the HA-ST22 and CA-ST22 strains, and the presence/absence of PVL supports the idea that these strains were of community origin [1,5,6].

Over the course of this study the frequency in which MRSA was recovered increased threefold, increasing from just 13 isolates in 2014 to 48 isolates in 2018. Whilst it is unclear as to the exact reason for the increase, optional screening of HCW and a quality improvement programme to improve adherence to screening for multi-drug resistant organisms across the hospital were introduced during the study period (April 2015 and October 2018 respectively). Both of which may have contributed to this increased recovery of MRSA. Pregnant women, often admitted in labour, are healthy and spend only a short period of time in the hospital, therefore lacking the typical risk factors associated with the acquisition and transmission of HA-MRSA. Despite this, the frequency of infection and colonisation with MRSA has increased among pregnant women and similarly, there is increasing frequency in the number of outbreaks in NICU reported. [6–8,15,24,25] During the period of the study, two outbreaks occurred from MRSA isolates of *spa* type t008. However, due to the anonymised nature of the patients included in this study, it is not possible to address modes of acquisition of MRSA for neonates. Elsewhere however, Staphylococcal colonisation (including MRSA) of mother and baby pairs has been shown to be common, with postnatal acquisition felt to be the mode of transmission. [26]. Widely applied strategies to prevent the spread of MRSA in NICUs include identifying colonized neonates and placing them on contact precautions, cohorting, hand hygiene for staff and parents and environmental cleaning [25].

Community-associated MRSA strains have been widely reported in Ireland and in particular, multidrug resistant strains ST1-MRSA-IV, ST772-MRSA-V and ST88-MRSA-IV have been linked to outbreaks in healthcare facilities. While ST1-MRSA accounted for 13.5% (23/171) of isolates in the current study, ST772 and ST88 were less frequently recognised. In addition, previously reported ST1 in Irish healthcare facilities harboured multidrug resistance genes, including those encoding mupirocin resistance [23]. However, only two isolates here exhibited mupirocin resistance. Previously reported ST772 in Ireland has been associated with an outbreak in a maternity hospital and also with numerous incidences of importation from people travelling to the Bengal Bay region where epidemiological links were identified with India [27]. In the current study, 39.7% (68/171) of isolates were recovered from patients with reported ethnicities other than Irish. The diverse population from which these isolates were recovered, along with the diversity exhibited among the MRSA collection suggests opportunities for further importation of MRSA into Ireland. Moreover, this

number may be higher as the ethnicity was unknown for almost a third of cases, included in this study.

Traditionally CA-MRSA strains exhibited less antimicrobial resistance due to the absence of the selective pressures seen in healthcare facilities. The ongoing reduction of HA-MRSA in hospitals since the late 2000s may have reduced the dissemination of HA-MRSA beyond healthcare settings, but has been associated with an expansion of CA-MRSA in the community setting [28]. In particular, single elements encoding multidrug resistance which are easily transferrable, and which resemble the accumulation of drug resistance seen in Gram-negative bacteria, have been described in MRSA [29]. Phenotypically, a wide range of resistance profiles were observed in the current study. However, molecular determination of resistance mechanisms cannot be achieved without the use of further molecular technologies such as DNA microarray and whole genome sequencing (WGS), therefore highlighting the importance of introducing such technology in enhancing current surveillance techniques.

There are clear limitations to this study. In many cases clinical and epidemiological data was lacking with the ethnicity of a large proportion of the study group unavailable, and limited information regarding underlying risk factors such as previous healthcare contact or travel abroad; therefore these factors were not considered in the current study. In addition, details of patients' history, including dates of admission were not available. Whilst epidemiological typing of the strains strongly support the suggestion that many of these strains are community associated, without such information it is not possible to confidently determine whether the MRSA was community or hospital acquired. Furthermore, *spa* typing lacks sufficient discriminatory power in outbreak situations and further characterisation of the isolates using whole genome sequencing could be useful for determining relatedness of isolates exhibiting indistinguishable *spa* types.

Conclusion

This study provides clear evidence of a diverse population of MRSA circulating in Ireland. In addition, it provides evidence that the population of MRSA in a maternity hospital in Ireland is considerably different from populations in other healthcare facilities. Many strains harbour multiple resistance and virulence genes which may provide a reservoir of MRSA to be imported into the hospital. These factors highlight the need for regular surveillance of MRSA in the patient population to ensure appropriate infection prevention and control interventions are in place at the earliest possible stage, and allow identification of possible importation events and outbreaks.

Credit author statement

Dr. Deirdre Broderick: Conceptualization; Methodology; Data Curation; Writing - Original Draft Preparation; Writing - Review & Editing Preparation; Visualization; Project administration. Gráinne Brennan: Conceptualization; Methodology; Resources; Data Curation; Writing - Original Draft Preparation; Writing - Review & Editing Preparation; Visualization; Supervision; Project administration. Dr. Brian O'Connell: Conceptualization; Writing - Review & Editing Preparation;

Supervision. Dr. Richard Drew: Conceptualization; Data Curation; Writing - Review & Editing Preparation; Supervision.

Conflicts of interest statement

The authors have no conflicts of interest to declare.

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