

Bacteriological Profiles and Antibiotic Susceptibility Patterns in Complicated Bacterial Infections of the Ears, Nose and Throat

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The objective of this study was to identify and map bacteriological profiles and antibiotic susceptibility patterns of complicated bacterial infections in the otorhinolaryngological (ENT) spectrum. Antibiotic resistance was another goal of our study. We defined complicated bacterial infections as: acute bacterial pathology of the ears, nose and throat with complications that required immediate surgical approach, chronic diseases of the ears, nose and throat that required surgical treatment due to ongoing bacterial infections, middle ear cholesteatoma with complications, complicated suppurated tonsillitis in children, intratonsillar abscess, parapharyngeal space abscess, cellulitis of the neck, face and/or involving cervical ganglia and neoplasms of the pharynx or larynx with an acute ongoing bacterial superinfection. The study was carried out over a period of 12 months, on a total number of 82 patients (children and adults), admitted in our department for the complicated bacterial diseases listed above. For the ENT spectrum, we uncovered that more than 50% of complicated bacterial infections were caused by 4 pathogens: Pseudomonas species, Staphylococcus aureus non-MRSA and MRSA species and Streptococcus species (other than Beta-haemolytic Group A Streptococci and Streptococcus pneumoniae). Almost two thirds of all complicated bacterial infections are complicated cases of sinusitis or mastoiditis that require immediate surgical treatment due to possible life-threatening complications.

Keywords: complicated bacterial infection, bacteriological profiles, antibiotic resistance, bacterial culture, bacterial antibiotic susceptibility.

Upper respiratory tract infections (URTI) are extremely frequent illnesses caused by an acute infectious process which involves the upper respiratory tract. In some cases, when certain more complicated URIs lack proper medical treatment, they may evolve into chronic diseases or may develop complications. Bacterial infections cause more complications and require antibiotic treatment, therefore a lot of studies have focused on describing bacterial profiles for organ-related infections, and the susceptibility of bacteria to current antibiotics in use. Although many studies on these topics have been carried out, the data is subjected to quick changes as the spectrum of bacteria is different from one country to another and even between different geographical regions in the same country. With the wide usage of antibiotics in the past decades and the emergence of many new resistant drug resistant bacteria, it has become necessary to constantly evaluate bacteriology reports obtained from URIs [1, 2, 3]. The purpose of this study was to investigate the current bacteriology profiles of complicated URIs treated in our ENT Department, to uncover antibiotic susceptibility patterns and also bacterial resistance to antibiotics. The clinical importance of most antibiotic related research is to contribute to existing antibiotic guides, therefore finding out which type of bacteria causes infections more often in the key anatomical sites of the ENT sphere (nose/sinuses, middle-ear) was another goal of this study.

Experimental part

Material and methods

This prospective study was carried out on 82 consecutive patients admitted for complicated URTIs in an ENT Department, Craiova Emergency County Clinical Hospital, between January 2017 and January 2018. We considered bacterial infections that required hospitalization and/or surgical treatment as “complicated URTIs”.

Bacteriology cultures were obtained differently depending on the site of the infection. Smears were prepared from the swab and stained by Gram stain. The swabs were cultured on a blood agar, MacConkey agar and chocolate agar plates. The plates were incubated overnight at 37°C. Colony count was obtained from blood agar plate. The growth of the organism was characterized by colony morphology and Gram's staining from the culture plates. A detailed biochemical testing was performed & antibiotic sensitivity testing was performed on Mueller-Hinton agar plates by Kirby-Bauer's disc diffusion method using suitable antibiotics as per CLSI (Central Laboratory Standard Institute) guidelines [3]. Aerobic and anaerobic cultures were tested for the collected specimen samples [4- 6]. Only cultures that revealed a moderate to heavy amount of bacterial growth were considered significant and included in this study. Fungi were excluded. Testing was also carried out for the Koch bacillus.

The antimicrobial agents tested were amikacin (AK), ampicillin (AM), amoxicillin/clavulanic acid (AMC), aztreonam (ATM), chloramphenicol (C), ceftazidime (CAZ), cefoperazone/sulbactam (CES), ciprofloxacin (CIP), clindamycin (CLI), clarithromycin (CLR), colistin (COL), ceftiofloxime (CPO), ceftriaxone (CRO), cefotetan (CTT), cefotaxime (CTX), cefuroxime (CXM), cefazolin (CZ), doxycycline (DO), erythromycin (ERY), ertapenem (ETP), cefepime (FEP), ceftazidime (FOX), gentamicin (GEN), high-dose gentamicin (GENHL), imipenem/cilastatin (IMC), imipenem (IPM), levofloxacin (LEV), linezolid (LNZ), meropenem (MEM), moxifloxacin (MXF), myconazole (MIC), nitrofurantoin (NIT), norfloxacin (NOR), ofloxacin (OFX), oxacillin (OXA), penicillin (P), rifampicin (RIF), tetracycline (TE), teicoplanin (TEC), tigecycline (TGC), tobramycin (TOB), piperacillin / tazobactam (TPZ), vancomycin (VA). To assure the accuracy and reliability of antimicrobial susceptibility test, the reference strains *S. aureus* (ATCC25923), *Escherichia coli* (ATCC25922), and *P. aeruginosa* (ATCC 27853) were used as internal quality controls.

Statistical Analysis. The gathered data were checked for accuracy, entered and analysed using SPSS Version 20 Statistical Software, Whonet 5.6 software (World Health Organisation, Geneva, Switzerland) and Microsoft Excel. Comparative analyses variables were made using the Student's t-test for two groups and one-way analysis of variance (ANOVA) for more than two groups. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

Results and discussions

Acute or chronic bacterial pathology of the paranasal sinuses with complications that required surgical approach accounted for 19 cases out (23.17%) of the total 82 cases admitted in the study. Acute or chronic middle ear disease, mastoiditis or complicated cholesteatoma that required surgical treatment accounted for 35 cases (42.68%). Complicated suppurative tonsillitis in children and intratonsillar abscesses accounted for 10 cases (12.19%). Parapharyngeal space abscesses accounted for only one case (1.219%). Cellulitis of the head and neck accounted for 10 cases (12.19%). Neoplasms of the pharynx and larynx with an acute ongoing bacterial superinfection accounted for 5 cases (6.1%), and chronic diseases of the ears, nose, pharynx or larynx with suspicious or abnormal local discharge accounted for 2 cases (2.43%).

Out of the total number of 82 patients, 52 (63.4%) were male, and 30 (36.6%) were female. Male patients seem more likely to develop complications from bacterial URTIs, and complications are more likely to occur in adults than in children (ANOVA, $P < 0.001$).

Prevalence of Isolated Bacteria from Cultures. We analysed a total of 95 antibiograms, collected from 82 patients included in our study. A total number of 12 (11.58%) tests came back negative. Out of all the positive results we were able to identify 14 different pathogenic bacteria species. Gram positive and negative bacteria were included. Among these bacterial species, the predominant bacterial isolates (over 50% of the isolated strains) were *Pseudomonas* species (13 samples – 15.66%) followed by *Staphylococcus aureus* MRSA (12 samples - 14.46%) and non-MRSA (10 samples – 12.05%) and *Streptococcus* species (11 samples – 13.25%). *Klebsiella* species and *Proteus* species followed with 7 detected samples each (8.43%). *Streptococcus pneumoniae* accounted for 6 samples (7.22%). 5 samples (6.02%) of *Enterococcus* species were detected. *Escherichia coli* and Gram Negative non-fermentative Bacilli accounted for 3 samples each (3.61%). Coagulase-negative *Staphylococci* and Group A Beta-haemolytic *Streptococcus* accounted for 2 samples each (2.4%). *Haemophilus influenzae* and *Acinetobacter* species were each detected in 1 sample (1.2%).

Antibiotic Susceptibility and Resistance of Isolated Bacteria. We documented 43 strains of gram positive bacteria (51.8%) and 40 strains of gram negative bacteria (48.2%). We considered the bacteria to be sensitive to a certain antibiotic if over 90% of tested strains were susceptible to the tested drug. We noted moderate sensitivity if over 50%

of tested strains were susceptible to the tested drug. We considered the bacteria to be moderately resistant if less than 50% of tested strains displayed susceptibility. Complete resistance to the drug was noted if 0% of tested strains were affected by the tested antibiotic. We used the same percentage indicator for the moderate sensitivity and moderate resistance of bacterial species as in the 'The European Database for antibiotic resistance. [7].

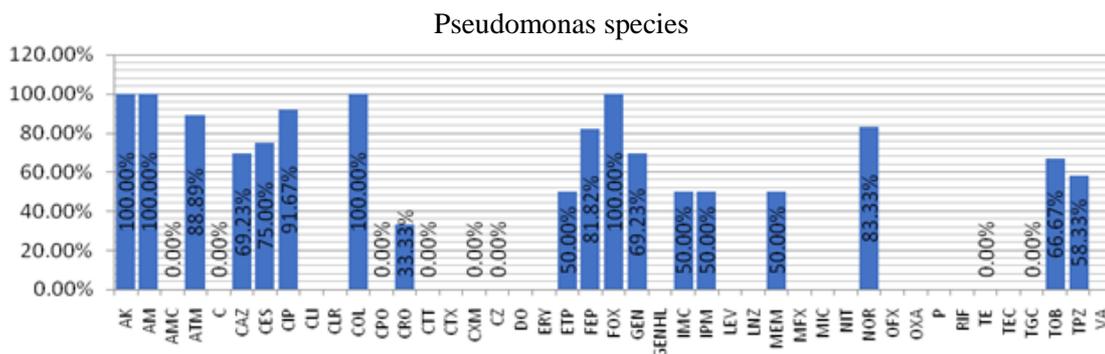


Chart 1. Antibiotic Susceptibility and Resistance for Pseudomonas sp.

The Pseudomonas species isolated were sensitive in over 90% of tested strains to: AK, AM, CIP, COL, and FOX. Pseudomonas sp. were moderately sensitive (sensitive in over 50% of tested strains but under 90%) to: ATM, CAZ, CES, FEP, GEN, NOR, TOB and TPZ. Pseudomonas was moderately resistant (50% or more of tested strains resistant to the drug) to: CRO, ETP, IMC, IMP, MEM. The strains were completely resistant (0.00% susceptibility) to AMC, C, CPO, CTT, CXM, CZ, TE and TGC.

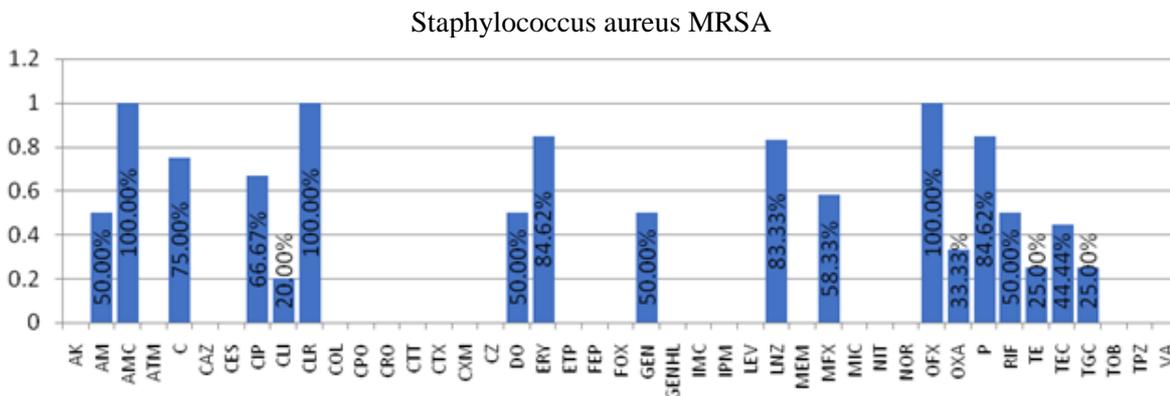


Chart 2. Antibiotic Susceptibility and Resistance for Staphylococcus Aureus MRSA

The isolated strains of Staphylococcus aureus MRSA were susceptible to the following antibiotics: AMC, CLR, OFX. The strains were moderately sensitive to: C, CIP, ERY, MFX, LNZ and P. We encountered moderate resistance towards: AM, CLI, DO, GEN, OXA, RIF, TE, TEC and TGC.

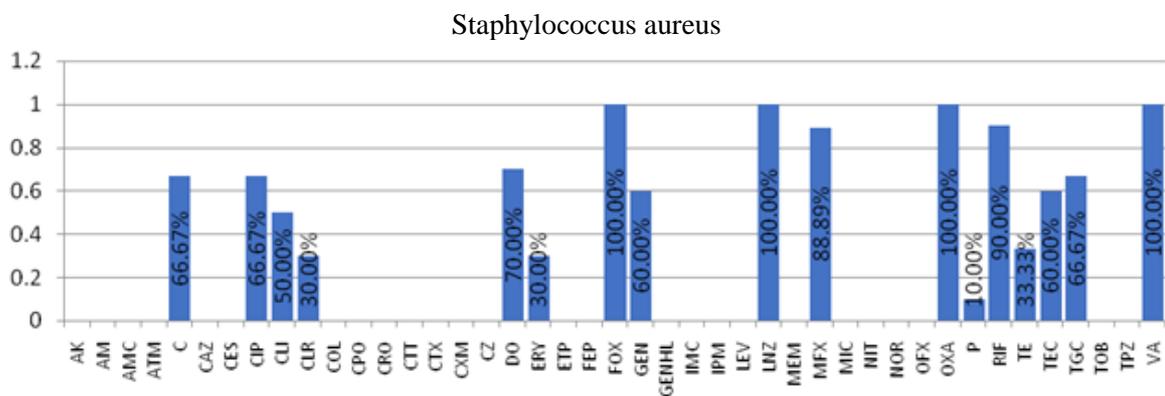


Chart 3. Antibiotic Susceptibility and Resistance for Staphylococcus Aureus non-MRSA

The isolated strains of Staphylococcus aureus non-MRSA were susceptible (sensitive in over 90% of tested strains) to the following antibiotics: FOX, LNZ, OXA, RIF and VA. The strains were moderately sensitive to: DO, MFX and RIF. We encountered moderate resistance (sensitive in less than 70% of tested strains) towards: C, CIP, CLI, CLR,

ERY, GEN, P, TE, TEC and TGC.

The isolated strains of *Klebsiella* sp. were susceptible to the following antibiotics: C, CIP, COL, CPO, CTX, ETP, FEP, IMC, IPM, OFX and TGC. The strains were moderately sensitive to: ATM, CAZ and CRO, GEN and TPZ. We encountered moderate resistance towards: CXM, CZ and TOB. *Klebsiella* sp. were fully resistant (0% sensitivity) to: AM, AMC, FOX and TE.

The detected strains of *Proteus* sp. were sensitive to: AK, CES, and MFX. They were moderately sensitive to: C, CIP, CRO, FEP, IPM, GEN, TPZ and TOB. Moderate resistance was uncovered towards: AM, AMC, ATM, CAZ, COL, CXM, CZ, ETP, FOX and TGC. Complete resistance of the bacteria towards CPO, CTX, IMC and TE was detected.

The other isolated species of *Streptococcus*, non-beta-haemolytic, were abundant, accounting for 13,25% of the total number of acute complicated bacterial infections. The isolated strains were sensitive to the following antibiotics: DO, FOX, GEN, IPM, LNZ, MEM, MFX, OFX, VA. Moderate sensitivity was registered towards: AM, C, CIP, CLI, CLR, CRO, CTX, P, TE and FEP. *Streptococcus* sp. was moderately resistant to: ERY, RIF, and TGC. No antibiotic susceptibility was detected for: OXA and TEC.

The most prevalent subspecies of *Streptococcus* was *Streptococcus pneumoniae*, detected in 6 cases of complicated bacterial infections (6,31%). This determined us to analyse its antibiotic susceptibility separately from the species family. *S. pneumoniae* was sensitive in 100% of tested strains to: C, CIP, CLI, CRO, DO, ERY, FEP, GEN, IPM, LNZ, MEM, MFX, MIC, NOR, OXA, RIF and VA. Moderate sensitivity was encountered for TE. Moderate resistance towards AM, CTX and P was noted. Resistance to CLR was also noted.

The *Enterococcus* species isolated were sensitive to: FEP, GEN, GENHL, LNZ, P, TEC and VA. The strains were moderately sensitive to AM, DO and CIP. Moderated resistance was noted towards: ERY and NOR. Resistance was encountered for CLI, CLR, NIT and OXA.

For the rest of the bacteria encountered in our study we consider the data as being insufficient for further analysis, as each strain was encountered in 3 or less cultures. These bacteria species are: *Escherichia coli*, Gram Negative Non-Fermentative Bacilli, Group A Beta-hemolytic *Streptococcus*, Coagulase-negative *Staphylococci*, *Haemophilus influenzae* and *Acinetobacter* species.

It is now common knowledge for medical researchers that antibiotic resistance is a natural phenomenon caused by the common use as well as abuse of antibiotic therapy both outside and inside of hospitals. Although the emergence of new, drug-resistant bacteria is inevitable, the transformation of bacterial strains into resistant strains and further into drug-resistant bacterial populations is greatly favoured by non-rational and excessive antibiotic therapy.

In order to limit the non-rational usage of antibiotics, Antibiotic Guidelines are constantly being published and updated. The use of an Antibiotic Guideline, and standardized antibiotic treatment schemes for each bacterial infection seems to be the only logical way to deter the appearance of new drug-resistant bacterial populations. In order to maintain the effectiveness of a guideline, constant reanalysis of bacterial diseases, both active strains and antibiotic susceptibility, is a requirement. Bacterial diseases with notable complications despite proper antibiotic treatment should be studied for etiological bacterial strains. Antibiotic susceptibility and resistance should ideally be noted in each case. Bacterial genomics are deemed very important when bacterial drug resistance is being taken into account [8].

Our study focused on complicated bacterial diseases that we encountered during the 12 month period of monitoring patients in our ward. These cases were admitted in the hospital, most of them having taken previous courses of antibiotics, and were more likely to uncover new changes in how the bacterial populations react to antibiotics. Gram positive and gram negative bacteria were both included in the study, and all statistically relevant strains were analysed. For the ENT spectrum of URTIs, we uncovered that more than 50% of diseases were caused by 4 pathogens: *Pseudomonas* species, *Staphylococcus aureus* (non-MRSA and MRSA included) and *Streptococcus* species (excluding beta-hemolytic group A streptococci and *Streptococcus pneumoniae*). The percentage of gram positive and gram negative bacteria did not show a statistically significant difference in favour of either germ populations. The complicated bacterial diseases we encountered during our study mostly involved the middle-ear or the paranasal sinuses. More than 60% of our study group patients had a bacterial infection involving one of those anatomical regions. The most frequent bacterial diseases in the ENT sphere that developed complications were acute and chronic otitis and mastoiditis.

Most complicated bacterial infections involving the pharynx (complicated suppurative tonsillitis in children, intratonsillar abscess, parapharyngeal space abscess) were caused by *Streptococcus* species (the most prevalent germ), *Staphylococcus aureus* non-MRSA and *Enterococcus*. The disease with the highest life-threatening risk in this group was the parapharyngeal space abscess, for which *Enterococcus faecalis* was responsible.

The complicated cases of cellulitis of the neck, face or involving cervical ganglia, were mainly caused by *Staphylococcus aureus* MRSA infections, followed by the non-MRSA *Staphylococcus*.

The acute and chronic bacterial pathology of the nose and paranasal sinuses that required immediate surgical approach, were mainly caused by germs as *Streptococcus* species and *Staphylococcus aureus* non-MRSA. However,

the third most identified germ in this diseases' group was the *Staphylococcus aureus* MRSA. The most common complication for this group of diseases was orbital cellulitis.

The middle-ear and mastoid bone pathology that required surgical treatment was caused by a plethora of germs, as we managed to isolate *Pseudomonas* species, *Proteus* species, MRSA and non-MRSA *Staphylococcus* species, *Streptococcus pneumoniae*, gram negative non-fermentative bacilli as well as *Klebsiella* species. The most prevalent of the germs isolated was however *Pseudomonas* species, followed by *Proteus* and MRSA *Staphylococcus* and *Klebsiella*. The most frequent complications that arose in this disease group were: meningitis, lateral sinus thrombophlebitis, and cerebral abscess.

In the case of *Pseudomonas*, known as a bacteria that easily colonizes hospital wards and medical equipment, the testing revealed curious results with susceptibility in over 90% of tested strains to amikacyn (AK), ampicillin (AM), ciprofloxacin (CIP), colistin (COL), and ceftazidime (FOX), and resistance to amoxicillin/clavulanic acid (AMC), chloramphenicol (C), ceftazidime (CPO), ceftazidime (CTT), ceftazidime (CXM), ceftazidime (CZ), tetracycline (TE), (tigecycline) TGC. We noted good susceptibility to ampicillin and ceftazidime, and resistance to second-generation cephalosporins. We attribute the good susceptibility to ceftazidime due to the cephamycins' structure based upon the cephem nucleus, granting efficiency towards anaerobic microbes. The curious spectrum of resistance and susceptibility may also be caused by different species of drug-resistant *Pseudomonas* and less resistant species, depending on their prior exposure to the hospital environment and to antibiotic treatment.

The isolation of *Staphylococcus aureus* MRSA from surgical wounds or cavities in the human body has been shown to be related to lower chances of primary healing due to complications after surgery [9]. The current method for testing MRSA is the resistance test to ceftazidime [10], which is also a surrogate test for resistance to oxacillin. The MRSA *Staphylococcus* may also colonize hospital wards and become difficult to control or eradicate. Its survival is promoted probably by the high usage of over the counter antibiotics, that can induce the transformation of methicillin susceptible *Staphylococcus aureus* (MSSA) in MRSA [11]. In our study, the prevalence of *Staphylococcus* MRSA was 14.46%.

Analyzing further, the problem is more complicated, as many patients are already colonized at admission with non-MRSA strains (MSSA included), mostly located in the pharynx [12]. Studies even show patients with acne, from which *Staphylococcus aureus* has been isolated [13]. Some of these patients may have already undergone abusive self-imposed antibiotic treatments, or even non-rational treatments prescribed by their general practitioner. These MRSA subpopulations can be selected through exposure to ceftazidime or oxacillin in up to 48 hours [11]. It is therefore difficult to place the moment in which a non-MRSA strain may convert into a MRSA strain, and even if that moment takes place inside or outside of the hospital.

As expected, the antibiotic susceptibility of both *Staphylococcus* MRSA and non-MRSA strains is highly different, with only 3 antibiotics accounting for sensitivities over 60% (moderately sensitive) in both types of bacterial populations: chloramphenicol (C), ciprofloxacin (CIP) and linezolid (LNZ). The expected low resistance to rifampicin (RIF), that most studies report, was not well-grounded with our findings in the ENT sphere, as only 50% of the *Staphylococcus* MRSA strains were sensitive.

The other most encountered type of bacteria in our study was the *Streptococcus* species. We classified the types of *Streptococcus* bacteria by species. Contrary to older data in literature, we came across little Beta-haemolytic Group A *Streptococcus* (2 samples accounting for 2.4% of all documented cultures), which we found to be of no statistical importance to our study. *Streptococcus pneumoniae* accounted for 6 samples (7,22%), and the most abundant types of *Streptococcus* were other species like *S. viridans* and *S. pyogenes*. Antibiotic susceptibility testing was carried out separately for the *Streptococcus* species (*S. viridans* and *S. pyogenes*) which were mainly identified in the pharynx and larynx, and separately for *Streptococcus pneumoniae*, which was identified in middle ear culture samples. *Streptococcus pneumoniae* was sensitive to most antibiotics tested, with moderate resistance to (CTX) and (P), and complete resistance to clarithromycin (CLR). The *Streptococcus* species (*S. viridans* and *S. pyogenes*) identified in the pharynx and sometimes in the larynx, displayed different patterns of susceptibility. Overall we may conclude that the *Streptococcus* species inhabiting the pharynx and/or larynx are more resistant to drugs, whereas *S. pneumoniae* which is mostly detected in samples collected from patients with otitis and mastoiditis, is still a highly antibiotic-sensitive bacteria. This difference may arise from irrational self-medication with antibiotics for common colds (a practice still common in Romania) or from the fact that most of acute tonsillitis or pharyngitis cases diagnosed by general practitioners rarely end up in being put on the appropriate course of antibiotics [14, 15].

Conclusions

Bacterial strains that cause infections with a high risk of complications in the otorhinolaryngological spectrum are certainly different between hospitals and regions alike..

Some bacterial strains isolated from our hospitalized patients have a higher resistance to certain antimicrobials, like higher-generation cephalosporins, linezolid or carbapenems, making the clinician doctor face a terrible dilemma when starting antibiotic therapy in unclear cases.

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