# STUDY ON THE ANTIBIOTIC RESISTANCE OF STRAINS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM ANIMALS WITH CONTAGIOUS ECTHYMA

## STUDIU ASUPRA FENOMENULUI DE ANTIBIOTICOREZISTENŢĂ A TULPINILOR DE STAPHYLOCOCCUS AUREUS IZOLATE DE LA ANIMALE CU ECTIMĂ CONTAGIOASĂ

Elisabeta MESTEŞANU[1),2)],
C. MESTEŞANU[3)], E. AVRAM[2)],
C. ANDOR[2)]

**ABSTRACT | REZUMAT**

Contagious ecthyma is a viral infectious disease that produces pustular vesicular rashes on the skin and mucosa, affecting a wide range of species, goats, sheep, wild ruminants, even carnivores and human.

In this paper, it were investigated the strains of *Staphylococcus aureus*, isolated from a contagious ecthyma outbreak, from the lesions caused by this virus in 5 flocks of sheep in 3 hotbeds of the disease. Attention was paid to the exact establishment of the type of isolated *Staphylococcus*, and the assessment of the antibiotic sensitivity by performing the antibiogram (CLSI, 2012). The results of the antibiogram showed an increased sensitivity to the action of Gentamicin (96,6%) and of quinolones (ciprofloxacin 86.6%, enrofloxacin 83.3%), even of trimethoprim (80%). *Staphylococcus aureus* is a ubiquitous germ, the cases of antibiotic resistance encountered due to the unusual use of antibiotics are frequently recorded in this germ. The increase of the penicillin resistance phenomenon, widely used in the past, has led to new generations of antibiotics in this group (semi-synthetic penicillin). Based on the sensitivity of the strains of *Staphylococcus aureus* isolated in 7 types of antibiotics, it was computed the MAR index (multiple antibiotic resistance index), in order to establish the risk degree represented by these types of strains for animals and humans. The values of the MAR index were between 0,14 and 0,57, in 63,3% of the cases the value exceeding the limit of 0,2, showing the possibility of the occurrence of the multiple antibiotic resistance. Moreover, the values over than 0,2 also show the fact that antibiotics are frequently used in the outbreaks of the disease investigated.

**Keywords**: contagious ecthyma, superinfection, *Staphylococcus*, antibiotic resistance, MAR index

Ectima contagioasă este o boală infecţioasă virală, care produce erupţii veziculo-pustuloase, pe tegumente şi mucoase, afectând o gamă variată de specii, caprine, ovine, rumegătoare sălbatice, chiar şi carnivorele şi omul.

În lucrarea de faţă, s-au investigat tulpinile de *Staphylococcus aureus*, izolate din 3 focare de ectimă contagioasă, din leziunile provocate de acest virus la 5 turme de ovine. S-a insistat asupra stabilirii exacte a tipului de stafilococ şi a evaluării sensibilităţii la antibiotice prin efectuarea antibiogramei. Rezultatele antibiogramei au arătat o sensibilitate crescută la acţiunea gentamicinei(96,6%) şi a quinolonelor (ciprofloxacină 86,6% şi enrofloxacină 83,3 %), chiar şi a trimetoprimului (80%). *Staphylococcus aureus* este un germen ubicvitar, cazurile de antibiorezistenţă întâlnite din cauza utilizării nejudicioase a antibioticelor fiind frecvent înregistrate la acest germen. Accentuarea fenomenului rezistenţă la penicilină, utilizată pe larg în trecut a dus la obţinerea unor noi generaţii de antibiotice din această grupă (peniciline semisintetice).

Pe baza sensibilităţii tulpinilor de *Staphylococcus aureus* izolate la 7 tipuri de antibiotice, s-a recurs la calcularea indicelui MAR (multiple antibiotic resistance index), pentru stabilirea gradului de risc reprezentat de aceste tulpini pentru animale şi om. Valorile obţinute ale indicelui MAR au fost între 0,14 şi 0,57, în 63,3% din cazuri valoarea depăşind limita de 0,2 ceea ce denotă posibilitatea apariţiei fenomenului de rezistenţă multiplă la acţiunea antibioticelor.

Totodată, valorile peste 0,2 indică şi faptul că în focarele de boală investigate se utilizează frecvent antibiotice.

**Cuvinte cheie**: ectimă contagioasă, suprainfecţie, stafilococ, antibiorezistenţă, indice MAR

Contagious ecthyma is a viral disease that affects predominantly domestic ruminants, wild animals but also pets (9). It is an infectious disease with acute evolution, produced by an epitheliotropic virus, clinically characterized by bladder rash, with predominantly buccal, podal, genital, mammary and ocular localizations (5, 9). The infection can also occur in humans, especially in people who handle diseased animals, manifesting in the form of hand or face rashes (7). The causal agent of this disease is a epitheliotrope virus, which contains DNA and is included in the *Poxviridae* family,

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine of Cluj-Napoca
2) Satu Mare Sanitary Veterinary and Food Safety Department
3) Santău Sanitary Veterinary Clinic, Satu Mare county
E-mail: mestesanu.elisabeta-sm@ansvsa.ro

the *Parapoxvirus* genre, known as "orf virus" or "contagious ecthyma virus", with sizes of 158 x 252 nm and morphology similar to the vaccine virus (9). The virus can also be found in the body of seemingly healthy animals, these being called carriers. In the infected crusts, it can withstand even 12 years and is resistant to glycerol and ether action (16).

The most serious complication of this disease is represented by secondary infections, which may lead to the worsening and extension of the disease's evolution, the result being even the death of the animal. In case of secondary infections, under the crust, the purulent collections can appear (2). Among the bacteria populating the lesions of contagious ecthyma are Staphylococci, Streptococci, *Escherichia coli*, *Fusobacterium necrophorum*, *Bacillus cereus*, *Corynebacterium spp.* Of the 28 species of the *Staphylococcus* genre, the most frequent species involved in the sheep pathology is *Staphylococcus aureus* causing a wide variety of predominantly suppurative disorders (14).

Antibiotic resistance testing has special indications especially for germs known to belong to microbial species that exhibit resistance to common antibiotics (1). The resistance mechanisms include the production of enzymes that inactivate the anti-inflammatory agent, target destruction, or blockage of the mode of action. An example is represented by the Gram-positive bacteria (*Staphylococcus aureus*) that produce intracellular β-lactamases - penicillinases - that degrade the β-lactam rings of penicillins (12). In the case of Gram-negative bacteria, the situation changes, besides the wide range of β-lactamases, to the acquiring of antibiotic resistance also contributes to the low permeability of the bacterial capsule or few penicillin receptors on the surface of the bacterial cell. Another situation is the presence of static methicillin staphylococci that lead to their remarkable resistance to all cephalosporin groups. Gram-negative bacteria can develop plasmid-mediated resistance to both penicillins and cephalosporins as well as to nitroimidazole antibiotics (8). Sulfamides and trimethoprim have lost their effectiveness in recent years due to the possibility of increased production of paraaminobenzoic acid but also by increasing the enzyme production that mediates the production of dihydrofolic acid, which decreases the affinity for this group of antibiotics. In order to prevent such phenomena, it was started the combined used of antibiotics, even if the associated antibiotic has no remarkable effects on the incriminated infectious agents, the combination triggers mechanisms that increase the curative effect (12).

As a consequence of the unproductive use of anti-infectives, the phenomenon of antibiotic resistance in the world of microorganisms has spread to alarming levels, thus becoming a major concern of medicine worldwide, concretized in the epidemiological monitoring of microbial antibiotic resistance (14). For these reasons, it was necessary to check the behavior of anti-infectives in all pathogenic microbial agents isolated from various sources (clinical human or animal cases, food, etc.). This study assessed the sensitivity to different antibiotics of *Staphylococcus aureus* strains isolated in contagious ecthymna outbreaks in Santău, Sudurău and Chereuşa localities in 2013.

## MATERIALS AND METHOD

The materials used are represented by crusts from suspicious lesions of bacterial superinfection. The crusts were harvested from 40 sheep from 5 flocks of sheep from Santău commune, from 3 outbreaks of disease. In 30 of the 40 samples it was isolated the *Staphylococcus aureus* as superinfection agent, i.e. 30 strains. The samples were harvested using sterile swabs imbedded in sterile physiological saline and stored in sterile containers containing sterile Amies transport medium(13) and transported, avoiding thermal shock or any other shock at LSVSA Satu Mare, where the determinations necessary for the elaboration of this study were made. In the first stage, the isolation of bacterial strains was performed and the usual culture mediums usually indicated by thioglycolate broth and selective Agar-agar were seeded. In the liquid medium, uniform turbidity was found, with a homogeneous deposit, and the solid medium was observed to have large, smooth, dense and creamy colonies (about 8 mm in size). To demonstrate coagulation activity, the coagulation test in the tube was performed. The morphological characteristics of the isolated bacteria were evidenced by the Gram staining on the smears, presenting them in the form of gram-positive coils placed in clusters. The biochemical characteristics of the strains isolated on API ID 32 Staphylococcus galleries were also tested, the results being read by using the miniAPi device.

Based on the tests performed, it was found that the isolated bacterium was *Staphylococcus aureus* in 30 of the 40 harvested samples. To the resistance to antibiotics, we used the Kirby-Bauer method, based on the presence or absence of an inhibition area whose diameter depends on the sensitivity of the tested bacterial strain (13). For the testing we used micro-ta-

blets that are available on the market in cartridges, such as: gentamicin (10µg), amoxicillin with clavulanic acid (20µg), cefuroxime (30µg), enrofloxacin (10 µg), ciprofloxacin 10µg) and trimethoprim (5µg), interpreting the results according to CLSI 2012.

## RESULTS AND DISCUSSIONS

In 30 of the total 40 samples harvested, there were isolated germs of the species *Staphylococcus aureus* according to the morphological features, cultures and biochemistry. Thus, after examination of the Gram stained smears (Fig. 1), in each sample the characteristics of gram-positive coci, unfolded in clusters, were observed.
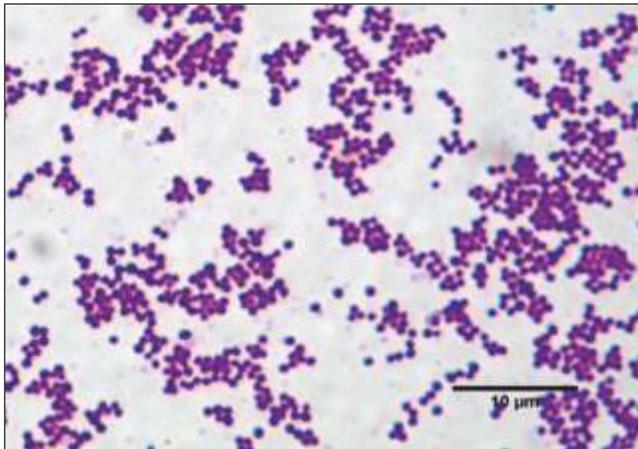


**Fig. 1. *Staphylococcus aureus*, Gram staining 1000x**

After the standard broth sowing according to the above method, moderate turbidity with a slightly homogeneous deposit was observed (Fig. 2).



**Fig. 2. Standard broth cultivation, comparatively with a sterile control sample**

In the passage on non-selective solid medium - blood agar, large, smooth, creamy colonies with large hemolysis zones were observed and on the selective medium (Baird Parker), there were seen some glossy black colonies, with white border, surrounded by a halo (Fig. 3).



**Fig. 3. *Staphylococcus aureus* colonies on selective Baird Parker medium**

The coagulase activity, given by staphylocoagulase, was also highlighted, which is an essential criterion in the identification of *Staphylococcus aureus*. We tested the sensitivity of the 30 strains of Staphylococcus Genus at the action of 7 antibiotics (Fig. 4).



**Fig. 4. Mueller Hinton agar, *Staphylococcus aureus*, antibiogram**

The standardized method has presented criteria for the interpretation of inhibition areas,through which it is possible to determine the level of sensitivity of the

**Table 1**

**The result of testing the action of the 7 antibiotic types on isolated *Staphylococcus aureus* strains, calculated by measuring the inhibition zone**

| Antibiotic<br><br>Sample no. | Genta-micin | Cefu-roxim | Amoxi-cillin with clavulanic acid | Enro-floxacin | Cipro-floxacin | Peni-cillin | Trime-thoprim |
|---|---|---|---|---|---|---|---|
| 1 | 19S | 18R | 17R | 22S | 22S | 23R | 18S |
| 2 | 18S | 23S | 23S | 25S | 25S | 20R | 17S |
| 3 | 17S | 26S | 25S | 22S | 26S | 19R | 12R |
| 4 | 19S | 21S | 26S | 25S | 25S | 21R | 20S |
| 5 | 20S | 23S | 14R | 25S | 25S | 21R | 21S |
| 6 | 20S | 23S | 22S | 21S | 27S | 22R | 20S |
| 8 | 19S | 25S | 24S | 14R | 14R | 16R | 9R |
| 10 | 18S | 21S | 14R | 19S | 26S | 10R | 20S |
| 11 | 18S | 21S | 24S | 14R | 14R | 21R | 21S |
| 12 | 19S | 24S | 23S | 14R | 14R | 25R | 18S |
| 13 | 19S | 23S | 15R | 21S | 25S | 12R | 19S |
| 14 | 18S | 25S | 25S | 20S | 27S | 20R | 18S |
| 15 | 20S | 21S | 25S | 17R | 17R | 21R | 18S |
| 17 | 21S | 27S | 25S | 20S | 25S | 22R | 17S |
| 18 | 21S | 25S | 23S | 16R | 16R | 19R | 20S |
| 19 | 18S | 13R | 26S | 21S | 27S | 18R | 21S |
| 20 | 19S | 18R | 17R | 23S | 24S | 19R | 20S |
| 21 | 20S | 22S | 17R | 23S | 23S | 23R | 19S |
| 22 | 19S | 21S | 23S | 20S | 25S | 21R | 9R |
| 23 | 19S | 22S | 21S | 21S | 23S | 23R | 10R |
| 25 | 18S | 22S | 25S | 22S | 22S | 23R | 9R |
| 27 | 20S | 21S | 26S | 23S | 25S | 23R | 13R |
| 28 | 20S | 26S | 25S | 20S | 25S | 26R | 20S |
| 29 | 18S | 25S | 23S | 21S | 23S | 26R | 19S |
| 30 | 18S | 25S | 24S | 24S | 24S | 21R | 19S |
| 33 | 19S | 23S | 21S | 24S | 16R | 20R | 17S |
| 34 | 19S | 23S | 21S | 22S | 22S | 18R | 17S |
| 37 | 13R | 11R | 21S | 25S | 25S | 17R | 18S |
| 38 | 20S | 22S | 21S | 25S | 25S | 20R | 18S |
| 39 | 17S | 25S | 18R | 23S | 23S | 21R | 12R |

various antimicrobial substances of the tested strains. These interpretation criteria were established by correlating the inhibition zones with the minimal inhibitory concentration. Their design was based on the study of a large number of bacterial germs, including those with known resistance mechanisms for certain classes of antimicrobial agents and in correlation with the pharmacokinetics of the antimicrobial substances used in therapy, as well as their clinical effectiveness. Depending on the diameter of the inhibition zone, the tested bacterial strain may be interpreted as being sensitive, moderately sensitive or resistant to the ac-

tion of the anti-microbians. The "sensitive" category implies that the test strains are inhibited by the usual concentrations of the antimicrobial agent, when used in the usual dose. The „moderately sensitive" category includes situations in which the minimal inhibitory concentration of the antibiotic reaches a certain blood and tissue level, but the response rate of the body may be lower than in the "sensitive" category. Substances included in the "moderately sensitive" category can be used in the therapy of localized infections, where the antibiotic is physiologically concentrated (e.g. quinolone and β-lactam in urine) or where it is possible to

Percentage representation of the sensitivity to each tested antibiotic

| Tested antibiotic | Genta-micin | Cefu-roxim | Amoxi-cillin with clavulanic acid | Enro-floxacin | Cipro-floxacin | Trime-thoprim | Peni-cillin |
|---|---|---|---|---|---|---|---|
| Sensitivity percentage | 96,6% | 86,6% | 76,6% | 83,3% | 86,6% | 80% | 0% |
| Resistance percentage | 3,4% | 13,4% | 23,4% | 16,7% | 13,4% | 20% | 100% |

use higher doses than usual (In the case of β-lactams). The "resistant" category means that the test strain is not inhibited by the usual systemic concentration achieved by the antimicrobial substance administered at the usual dosage or the test strain possesses specific resistance mechanisms (e.g. β-lactamases); in such cases, therapy is ineffective. In all cases, isolated *Staphylococcus* strains were found to be resistant to penicillin action, the inhibition zone being less than 28 mm, indicating the existence of β-lactamase. For the rest of the tested antibiotics the results were satisfactory, most of the isolated strains being susceptible to the action thereof. It was found that 29 of the 30 isolated strains proved to be sensitive to the action of gentamicin. It was also tested the sensitivity to cefuroxime, finding that 26 strains were sensitive to the activity thereof. In the case of amoxicilin with clavulanic acid, 23 of the 30 isolated strains proved to the sensitivity thereof. Enrofloxacin proved to be effective only for 25 strains, while ciprofloxacin was effective for 26 strains. 24 strains proved to be sensitive to trimethoprim. In Table 1 are represented the diameters of the inhibition areas for each tested strain and antibiotic.

Following the comparison of the results obtained, we found that in 19 of the 30 isolated strains, antibiotic resistance was multiple, these being resistant to 2, 3 or even 4 antibiotics tested, representing 63,33% of total number of isolated. 20% of the isolated strains were resistant to 3 of the antibiotics tested, 23,33 % in 2 of the antibiotics, 3,33 % in 4 of the antibiotics, 53,34% in one antibiotic. The best results were obtained when testing Gentamicin, of the 30 strains being only one resistant, although the literature described the resistance to to aminoglycosides of the *Staphylococcus aureus* (12). Similarly, trimethoprim and, in generally, sulfamides are considered antibiotics with low efficacy in the treatment of staphylococcal infections but our results showed a sensitivity of 80 % (7, 12). Compared to the rest of the antimicro-

bials tested the amoxicillin-clavulanate combination were low as it is shown in Tables 1 and 2. Expressed as a percentage, the result of the antimicrobial test demonstrates that except for penicillin, all the other 6 antibiotics tested were found to be effective in the event of a contagious ecthyma complication, the results being higher than 70% (Tables 1 and 2).

Resistance to antibiotics is a problem representing a major concern in veterinary medicine and human medicine because many cases of staphylococcus have been found to be resistant to antibiotics. In this regard, after performing the antibiograms and the interpretation of the results thereof it was established the MAR index (multiple antibiotic resistance index) to determine whether or not the disease outbreaks of which they are isolated represent a major risk source.

The MAR index represents the ratio between the number of antibiotics to which the isolated *Staphylococcus* of each sample is resistant and the total number of antibiotics used (1, 7, 10). The index was computed for each *Staphylococcus* and the results obtained are presented in Table 3.

In 19 of the 30 isolated strains, the MAR index was higher than 0,2, which represents a percentage of 63,33%. This situation reflects that multiple antibiotic resistance occurred because at the values of 0,2 indicates a resistance to 2 antibiotics. According to Jorgensen et al. (6) and Obajuluwa et al. (11), the value of 0,2 is a high value for the MAR index. This, according to Adegoke and Mkize et al. (1), it means that there is a high risk of occurrence of the multiple antibiotic resistance phenomenon and that the isolated bacteria come from areas where anti-microbians are often and unjustifiably used. The highest possible value of MAR index is 1, that indicates a resistance to all the tested antibiotics, in our case we did not get such result, but the 8 samples with the value of 0,42 and the one with 0,57 represented a reason to concern.

**Table 3**
**MAR index – multiple antibiotic resistance**
**for every sample**

| Sample no. | MAR Index |
|---|---|
| 1 | 0,42 |
| 2 | 0,14 |
| 3 | 0,28 |
| 4 | 0,14 |
| 5 | 0,14 |
| 6 | 0,14 |
| 8 | 0,57 |
| 10 | 0,28 |
| 11 | 0,42 |
| 12 | 0,42 |
| 13 | 0,28 |
| 14 | 0,14 |
| 15 | 0,42 |
| 17 | 0,14 |
| 18 | 0,42 |
| 19 | 0,28 |
| 20 | 0,42 |
| 21 | 0,28 |
| 22 | 0,28 |
| 23 | 0,28 |
| 25 | 0,28 |
| 27 | 0,28 |
| 28 | 0,14 |
| 29 | 0,14 |
| 30 | 0,14 |
| 33 | 0.28 |
| 34 | 0.14 |
| 37 | 0.42 |
| 38 | 0.14 |
| 39 | 0.42 |

## CONCLUSIONS

Most of the secondary infections occurred in the 3 outbreaks of contagious ecthyma are those with *Staphylococcus aureus*, this being the major cause of the complications occurred, such as the various dermatitis, or mammitis, gingivitis or palatinitis or other purulent processes. This is also due to the fact that Staphylococci are ubiquitous germs, even on the skin or mucous membranes (6, 14).

Given the frequency of secondary infections, it was required the isolation and identification of the causative agent of the complications to establish a correct treatment.

Most of the antibiotics tested proved to be efficient, therefore it was not difficult to choose the antibiotic required to establish the anti-infective treatment in the 3 outbreaks. Contrary to the information in literature (3, 12), high percentage of trimethoprim resistance was found although it is known that mecha-

nisms by which production of para-amino-benzoic acid increases and changes in the enzyme increases the di-hydro-folate acid, decreases the affinity to trimethoprim.

The fact that the penicilin resistance was signaled in all the isolated strains show the fact that the activity β-lactamase of the isolated strains was present. Since penicillin is an affordable and widely used product in veterinary medicine, the result obtained could be expected.

Following the tests performed, it was found an increased sensitivity of the isolated *Staphylococcus* to the action of the products of the category of quinolones and gentamicin. In our case, the ability to inhibit the 30S ribosome is effective, as described in the literature (3, 11).

The high computed values of the MAR index in most of the cases show the fact that the possibility of the occurrence of resistance to the multiple antibiotic was high in the 3 outbreaks of disease and a risk for the contacts.

## REFERENCES

1. *Adegoke A.A., Okoh A.I,* (2014), Species diversity and antibiotic resistance properties of *Staphylococcus* of farm animal origin in Nkonkobe Municipality, South Africa, Folia Microbiol (Praha). 59(2): 133–140
2. *Cargnelutti J.F. , Masude E.K. , Martins M. , Diel D. G. , Rock D.L. , Weiblen R. , Floeres E.F. ,* (2011). Virological and clinico-pathological features of orf virus infection in experimentally infected rabbits and mice, Microbial Pathogenesis. 50(1):56-62
3. *Deyno S., Toma A., Worku M,  Bekele M,* (2017), Antimicrobial resistance profile of Staphylococcus aureus isolates isolated from ear discharges of patients at University of Hawassa comprehensive specialized hospital. BMC Pharmacology and Toxicology, BMC series DOI: 10.1186/s40360-017-0141-x.
4. *Eliopoulos G.M., Jana M. Swenson, Janet A. Hindler, MCLS, Maria M. Traczewski, BS, Jenkins S.G., Turnidge J.D, Lewis J.S II, Weinstein M.P., Limbago B, Barbara L. Zimmer,* (2012), CLSI Standards , Volume 35, Number 1.
5. *Haig D.M.,* (2006). Orf virus infection and host immunity. Curr Opin Infect Dis.
6. *Jorgensen J.H., Pfaller M.A., Karen Carroll, Funke G., M.L. Landry, Sandra S. Richter, Warnock D.W.,* (2015). Manual of Clinical Microbiology, 11th Ed.

7.  *Knipe D.M., Diane E. Griffin, Lamb R.A., Straus S.E.,Howley P.M.,Martin M.A., Roizman B.,* (2007), Fields ˙ Virology, Fields Virology, 5th edition, Vol.2, Volters Kluwer-Lippincott Williams and Wilkins.

8.  *McVey D.S., Melissa Kennedy, Chengappa M.M.,* (2013), Veterinary Microbiology, Wiley-Blackwell.

9.  *Moga R.M.,* (2005). Boli virotice şi prionice ale animalelor, Ed. Brumar, Timişoara, pp 201-205.

10. *Nworie A., Elom M.O.,Gideon I.A, Azi S.O., Okekpa S.I., Ukwah Victor B.N., Usanga U, Okon U.N., Chinwe E., Olayinka B.O., Onaolapo J.A., Ehinmidu J.O.,* (2017), Multi-drug resistant Staphylococcus aureus from poultry farms in Ebonyi state, Nigeria. International Journal of Microbiology, Genetics and Monocular Biology Research, Vol.2, No.3, pp.1-11.

11. *Obajuluwa A.F., Olayinka B.O., Adeshina G.O., Igwe J.C.and Onaolapo J.A.,* (2015). Antimicrobial Susceptibility Pattern of Staphylococcus aureus Isolates from Orthopaedic Patients in Abuth, Zaria.

12. *Quinn P.J., Markey B.K, Leonard F.C., Fitzpatrick E.S., Fanning S., Hartigan P.J.,* (2011), Veterinary Microbiology and Microbial Disease, John Wiley and Sons Ltd,Publication 19(2):127–131.

13. *Rafila A.,* (2008). Medii de cultură. Medii de transport şi conservare. Tratat de microbiologie clinică, ediția a II-a, sub redacția Buiuc D., Negu M., Ed. Medicală

14. *Felicia Toma Săcărea,* (2006). Bacteriologie Medicală, Ed. University Press, Târgu Mureş.

15. *Subramani S., Vignesh S.,* (2012). MAR Index Study and MDR Character Analysis of a few Golden Staph Isolates. Asian Journal of Pharmacy and Life Science Vol. 2 (2), 151

16. *http://www.merckmanuals.com/vet/integumentary_system/contagious_ecthyma/overview_of_contagious_ecthyma.html*