[2] *Universiti Sultan Zainal Abidin, Centralised Laboratory Management Centre, Besut, Terengganu, Malaysia*
[3] *Universiti Putra Malaysia, Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Serdang, Selangor, Malaysia*

Purpose: *Staphylococcus aureus* is an opportunistic bacterium that causes a wide range of infections. Recently, the emergence of multidrug-resistant *Staphylococcus aureus* (MDRSA) in animal farming industry has become an increasing public health concern worldwide. Nevertheless, data regarding the genotypes of MDRSA isolated from occupational livestock exposure is limited. The objectives of this study were to identify and characterize the dominant staphylococcal protein A (*spa*), accessory gene regulator (*agr*) and immune evasion cluster (IEC) types of MDRSA isolated from livestock veterinarians and animal farm workers in Malaysia.

Methods & Materials: A total of 30 MDRSA isolates were involved in this study. The MDRSA were previously isolated from livestock workers with constant exposure to animals. *S. aureus* isolates were confirmed via polymerase chain reaction (PCR) amplification of the *nuc gene*. All 30 isolates were categorized as multidrug-resistant (MDR) strains using the disk-diffusion assay. gDNA of the bacteria isolates was extracted using simple boiling method. The genotypes of the MDRSA were confirmed using *spa* and *agr* typing. PCR detection of IEC, including *scn*, *sak*, *chp*, *sea* and *sep* genes were carried out to determine the IEC types of MDRSA isolates.

Results: A total of 17 different *spa* types were detected among the 30 MDRSA, with t4171 (16.7%) and t189 (16.7%) been the predominant *spa* types. 66.6% of MDRSA belong to *agr* I while 33.3% were from group II. Meanwhile, the predominant IEC type was type E (16.7%).

Conclusion: This study demonstrated the presence of diverse *spa* types, suggesting genetic diversity among the MDRSA isolated from livestock workers. MDRSA from *agr* I and IEC type E were prominent as compared to other genotypes.

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PS01.02 (481)

A Sustainable Optimized Native Lysostaphin Production and Immunopurification Approach for fighting the Problematic Resistant Super Bug Staphylococcus aureus

H. Nour El-Din [1,*], M. Hussein [1,2], A. Attia [1,2]

[1] *Faculty Of Pharmacy Cairo University, Microbiology and Immunology, Cairo, Egypt*
[2] *School of Pharmacy, New Giza University, Giza, Egypt*

Purpose: Antibiotics, the greatest twentieth century weapon, are faced with a repertoire of resistant microorganisms that lowers their control over life-threatening infections. Hence, mining different natural products for antimicrobial activity has been an attractive approach to combat problematic microorganisms such as the super bug, *Staphylococcus aureus*. Herein, we propose an optimized production and immunopurification approach for the production of the native staphylococcal cell-wall degrading enzyme "lysostaphin (LST)". We are presenting this approach as a sustainable platform that would further help circumvent the antibiotic resistance global issue.

Methods & Materials: Native LST production from *S. simulans* cultures was optimized using full factorial design, evaluating the influence of four independent factors (temperature, agitation, media:air ratio, and growth time) against the dependent variable of LST yield measured in unit activity (U)/mg dry weight. The activity of the optimized native LST (N-LST) was tested against a collection of clinical methicillin-resistant *S. aureus* (MRSA) isolates. Recombinant LST, produced locally at our laboratory, was then used as an immunogen for polyclonal antibodies production in BALB/C mice. Finally, the produced anti-LST IgGs were purified and assessed using enzyme-linked immunosorbent assay (ELISA) and then attached to CNBR-activated sepharose beads to be used for N-LST purification.

Results: N-LST optimized production yielded up to 5.6 U/mg dry weight of the native enzyme which is significantly more (153%) than the previously reported yield. The enzyme was also found to be active against clinical MRSA isolates. The immunoaffinity purification of the N-LST was successful and yielded highly active enzyme.

Conclusion: The current work identified conditions for N-LST production that yielded a highly active product, surpassing the reported methods. We also offer a sustainable purification platform incase higher grade of the enzyme was required. The current study serves the ongoing efforts to combat the surge in antimicrobial resistance and establishes a good start for the adoption of N-LST production systems to satisfy the research needs in developing countries.

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PS01.03 (118)

Phylogenetic Diversity and Susceptibility of Candida Species from Women using Contraceptive Devices in Central Nigeria

A. Lillian [1,2], A. Chuku [1], N. Joseph [1], B. Ajide [3], R. Reuben [4,*], O. Akwashiki [5]

[1] *Federal University of Lafia, Department of Microbiology, Lafia, Nasarawa, Nigeria*
[2] *Bingham University, Department of Biological Sciences, Karu, Nasarawa, Nigeria*
[3] *Bingham University, Department of Biological Sciences, Karu, Nasarawa, Nigeria*
[4] *German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany*
[5] *Federal University of Lafia, Department of Zoology, Lafia, Nasarawa, Nigeria*

Purpose: The use of contraceptive devices predisposes women to vulvovaginal candidiasis globally. Despite the high incidence of vulvovaginal candidiasis and antifungal resistance to azoles, the genetic diversity and resistance pattern among contraceptive users in Nigeria is poorly investigated. This study therefore sought to characterize and determine the phylogenetic breadth of *Candida* spp. as well as their resistance to antifungal agents from women using contraceptive devices in central Nigeria.

Methods & Materials: This study recruited 1,600 women using contraceptive devices that visited gynaecology and obstetrics clinics in central Nigeria between August, 2018 to February, 2020. *Candida* spp. were isolated and characterized using conventional methods and the sequencing of the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA). Bayesian phylogenetic analysis was used to characterize the diversity of *Candida* spp. Agar well diffusion technique and primer-specific PCR were used for the determination of antifungal susceptibility profiles and the presence of resistant genes.

Results: Five Candida spp. were identified from 710 contraceptive users with vulvovaginal candidiasis. Although Candida albicans was the predominant (43.23%) *Candida* spp. isolated, other non-albicans *Candida* spp. included C. glabrata (19.01%) C. tropi-

calis (15.77%) C. *parapsilosis* (8.87%) and C. *akabanensis* (13.09%) respectively. The molecular characterization of the different *Candida* spp. and their phylogenetic relationships were confirmed using the Bayesian analysis. All the *Candida* spp. revealed varying degrees of susceptibilities to voriconazole, fluconazole and nystatin. However, C. albicans showed 29.0% resistance to fluconazole, *C. tropicalis* showed 46.0% and 14.0% resistance to nystatin and voriconazole while *C. akabanensis* showed 100% resistance to voriconazole and fluconazole, respectively. Kruskal-Wallis Chi-square test using 'R' (Version 3.2.2) showed nystatin as the most effective antifungal agent (Kruskal-Wallis $\chi^2 = 786.03$, df = 2, $P < 0.001$) against *Candida* spp. Also, resistant gene *Erg11* was identified in all the *Candida* spp. resistant to the antifungal agents tested.

Conclusion: Women using contraceptive devices in central Nigeria harbour phylogenetically diverse *Candida* spp. including *C. akabanensis* an uncommon cause of vulvovaginal candidiasis. Out of these *Candida* spp. *C. albicans, C. tropicalis* and *C. akabanensis* are notable for multidrug drug resistance as well as harbouring resistant gene *Erg11*.

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Multi-strain Probiotics Upregulated Anti-inflammatory Properties and Reduced Pasteurella multocida Mortality in Broilers

R. Reuben [1,2,*], S. Sarkar [1], H. Ibnat [1], M.A. Ahasan Setu [1], P. Roy [1], I. Jahid [1]

[1] *Jessore University of Science & Technology, Microbiology, Jashore, Bangladesh*
[2] *German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany*

Purpose: *Pasteurella multocida* is the highly contagious and zoonotic pathogen of a broad range of animal diseases causing devastating ecological and economic challenges globally. While about 80.5% of *P. multocida* infections have shown high degree of resistance to broad range of commonly used antibiotics, dietary inclusion of probiotics may become a suitable alternative in mitigating *P. multocida* infections in animals, hence reducing human spread as well as safeguarding the environment. It was hypothesized that dietary supplementation with multi-strain probiotics consisting of *Lactobacillus plantarum*, *L. fermentum*, *Pediococcus acidilactici*, *Enterococcus faecium* and *Saccharomyces cerevisiae* would mitigate *P. multocida* infection in broilers as well as improving gut health, haemato-biochemical parameters and growth performance.

Methods & Materials: A total of 120 birds were randomly allocated to 6 treatments with 2 replicates each, and were fed with a basal diet supplemented with multi-strain probiotics (10^8 CFU/kg) and then orally challenged with 10^8 CFU/mL of *P. multocida*. Clinical manifestations of *P. multocida* infection and mortality were recorded as well as the expression of anti-inflammatory genes, haemato-biochemical parameters, gut microbiota and growth performance.

Results: Probiotics supplementation significantly ($P < 0.05$) improved growth performance and feed efficiency as well as reducing ($P < 0.05$) the population of intestinal *P. multocida*, enterobacteria, and mortality. Haemato-biochemical parameters including total cholesterol, white blood cells (WBC), proteins, glucose, packed cell volume (PCV) and lymphocytes improved ($P < 0.05$) among probiotics fed birds when compared with the controls. Also, transcriptional profiles of anti-inflammatory genes including hypoxia inducible factor 1 alpha (HIF1A), tumor necrosis factor- (TNF) stimulated gene-6 (TSG-6) and prostaglandin E receptor 2 (PTGER2) in the intestinal mucosa were upregulated ($P < 0.05$) in probiotics fed birds.

Conclusion: The dietary inclusion of the multi-strain probiotics improves growth performance, feed efficiency and intestinal health while attenuating inflammatory reaction, clinical signs and mortality associated with *P. multocida* infection in broilers, and these suggest that the probiotics can be used as alternative against *P. multocida* infections.

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PS01.05 (495)

Antimicrobial Resistance Dynamics in the Chicken Gut after Amoxicillin and Thiamphenicol Treatments

A. Laconi [1,*], R. Tolosi [1], M. Cuccato [2], L. Mughini Gras [3], F.T. Cannizzo [2], G. Tilli [1], A. Piccirillo [1]

[1] *University of Padua, Department of Comparative Biomedicine and Food Science, Legnaro, Italy*
[2] *University of Turin, Department of Veterinary Science, Turin, Italy*
[3] *Utrecht University, Department of Population Health Sciences, Utrecht, Netherlands*

Purpose: the aim of the study was to investigate the selective pressure exerted by amoxicillin (beta-lactam) and thiamphenicol (phenicol) administration on the abundance of antimicrobial resistance genes (ARGs) in the chicken gut.

Methods & Materials: eighteen broiler chicks were allocated in three groups and reared without treatment or treated with either amoxicillin or thiamphenicol at 5 days of age for three consecutive days. Cloacal swabs were taken from all birds at 1 day of age, as reference, and then on days 8, 19, and 28. At the end of the rearing cycle, birds were slaughtered and the caecal content was aseptically collected. Swabs (n = 72) and caecal contents (n = 13) were analysed by quantitative PCR assays to generate data on the abundance of fourteen ARGs conferring resistance to beta-lactams (n = 8) and phenicols (n = 6). Difference in ARGs abundance over time points within the same group was assessed using Friedmann test with Dunn's test for multiple comparisons, while Spearmann's rank correlation was used to assess the co-occurrence of beta-lactam and phenicol ARGs.

Results: increased abundance of ARGs conferring resistance to phenicols was observed in the groups treated with either amoxicillin or thiamphenicol ($p < 0.05$). In detail, the abundance of *floR* and *cmlA* genes was significantly increased ($p < 0.05$) after treatment with either of the two antimicrobials up to 21 days post-treatment (d.p.t.). Amoxicillin treatment enhanced the abundance of bla_{SHV} at 1 d.p.t. ($p < 0.05$). Positive correlations between bla_{TEM-1} and *floR* ($p < 0.05$; $r = 0.245$) and *cmlA* ($p < 0.0001$; $r = 0.325$) were also observed.

Conclusion: the results of the study seem to suggest an increasing trend of abundance of bla_{TEM-1}, conferring resistance to beta-lactams, and *cmlA* and *floR*, conferring resistance to phenicols, under the selective pressure exerted by amoxicillin and phenicols.

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