SULPHONAMIDE DRUGS AND *PSEUDOMONAS AEROGINOSA* RESISTANCE: A REVIEW

Egbujor Melford C.*[1], Nwobodo David C.[2], Egwuatu Pius I.[2], Abu Ifeanyichukwu P.[2], Ezeagu Casmir U.[3]

[1]Department of Industrial Chemistry.
[2]Department of Microbiology.
[3]Department of Biochemistry, Renaissance University, Ugbawka, Enugu State, Nigeria.

ABSTRACT

Sulphonamide drugs were the first antimicrobial agents to be used systematically which have also been widely utilized as antimalarial, anticancer, antiretroviral, diuretic, antihypertensive agents to mention but a few. Sulphonamide was first isolated from coal tar in 1935 for the treatment of bacterial infections due to its selective toxicity against bacterium cell thereby paving the way for the antibiotic revolution in medicine. Over the years, sulphonamide drugs lost its preference as the first line drug of choice in the treatment of bacterial infections because of the increased resistance exhibited by certain bacteria especially *Pseudomonas aeruginosa*. The unique resistance of *Pseudomonas aerusginosa* against sulphonamide is quite worrisome due to the fact that this bacterium being one of the scariest bacteria in the world is responsible for several serious infections that were erstwhile curable with inexpensive sulphonamide drugs. Several isolates of *Pseudomonas aeruginosa* have considerable defense against reliable antibiotics and their concomitant infections are difficult to treat because of complex enzymic and mutational mechanisms of resistance exhibited by *Pseudomonas aeruginosa*. This review observed and proposed the potential ways of tackling the recalcitrance of *Pseudomonas aeroginosa* to sulfonamide though structural modifications and derivatization.

KEYWORDS: Sulphonamides, Pseudomonas Aeruginosa, Antimicrobial Resistance.

INTRODUCTION

Sulphonamides were the first class of antibiotics to be discovered and put into clinical use.[1] The combination of biologically active sulphonyl and amine moieties makes sulphonamides the basis of several groups of drugs.[2] They have been extensively studied for their chemotherapeutic activities as antimicrobial, antimalarial, antileprotic and antioxidant agents.[3-4] Sulphonamides are notable for their broad spectrum antimicrobial activities against many gram-negative and gram-positive microorganisms. They were found to be bacteriostatic and therefore do not kill the bacterium but inhibit their growth and multiplication.[5] Sulphonamide drugs are known to be the oldest and most widely used antibiotics in animal treatment due to the fact that they are relatively cheap and efficacious in the treatment of several microbial infections in veterinary medicine.[6] Clinically, aliphatic sulphonamides have been extensively utilized in the treatment of chronic urinary tract and gastrointestinal infections.[7] Aromatic and heteroaromatic sulphonamides having carbonic anhydrase inhibitory ability are useful antitumor agents.[8-10] The versatility of sulphonamide as a pharmaceutical compound can be seen in their usefulness in the treatment and prevention of disease syndromes such as occidiosis, toxoplasmosis, actinobaillosis, metritis, respiratory infections and mastitis.[11-13]

Pseudomonas aeruginosa is a ubiquitous rod shaped gram-negative bacterium which is responsible for several bacterial infections in plants, animals and humans.[14] It is notable for its multidrug resistance especially against sulphonamide drugs due to its inherently advanced antibiotic resistance mechanisms that make treatment very difficult.[15] *Pseudomonas aeruginosa* is considered an opportunistic, nosocomial microbe of immune compromised individuals that causes serious blood, urinary tract, airway, wounds, burns infections, to mention but a few.[16] *Pseudomonas aeruginosa* is the causative agent for about 10-5% of nosocomial infections globally.[17] And these infections are hard to treat because *Pseudomonas aeruginosa* has the peculiar ability of exhibiting natural and acquired mechanisms of resistance to several groups of antimicrobial agents especially sulphonamide drugs.[18-19] In plants, *Pseudomonas aeruginosa* causes soft rot such as *Arabidopsis thaliana* in thale cress,[20] and Lactuca sativa in lettuce.[21,22] while in invertebrates, it is responsible for *Caenorhabditis elegans* in nematode,[23-24] Drosophilia in the fruit fly,[25] and Galleria mellonella in moth.[26]

## Resistance to Sulphonamide; a Matter of Concern

It is worrisome that, in spite of the fact that sulphonamides are very cheap, useful and effective pharmaceutical compound that exhibit wide range of biological activities, they are rapidly losing relevance because of the emergence of resistant and low antibiotic susceptible *Pseudomonas aeruginosa*,[27] which is responsible for a death rate ranging from 18% to 61%.[28-30] Numerous studies have focused solely on the resistance of pathogenic microbes against sulphonamide but only few have been reported about the possible ways out of the menace. This review therefore explores the antimicrobial resistance and possible ways of addressing the perennial challenge of pseudomonas aeruginosa recalcitrance against sulphonamide drugs.

Synthesis of Sulphonamides

Sulphonamides are synthesized in numerous ways. Koburger and Forster in 1903 and 1914 respectively ultilised simple alkyl sulphonyl chlorides in the synthesis of the earliest sulphonamides [31-32]. The generally accepted approach in recent times involves the reaction between sulphonyl chloride and a primary or secondary amine. Sulphonyl chlorides are generally synthesized by oxidation of the required thiol simply by bubbling chlorine gas into the reaction.[33]

$$RSO_2Cl + R_2NH \longrightarrow RSO_2NR_2 + HCl$$

Laudadio *et al*,[34] reported a synthetic approach for sulphonamides *via* an eco-friendly electrochemical method that facilitated the oxidative coupling of primary amines with thiols as a classic approach.

$$RSO_2Cl + R_2NH \xrightarrow{base} RSO_2NR_2 + HCl$$

$$\uparrow$$

$$R\text{-}SH$$
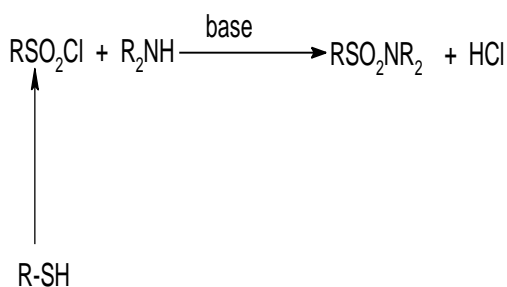
Mode of Action of Sulphonamide Drugs

The sulphonamide drugs in their peculiar mode of action is governed by the principle of selective toxicity which exploits some metabolic differences between mammalian cells and bacterial cells. Generally, folic acid is essential for the growth of cells. Folic acid being a vitamin in food is diffused or transported into human cells. Conversely, folic acid does not permeate bacterial cell walls by either diffusion or active transport and as a result bacteria must depend on synthesized folic acid from *p*-aminobenzoic acid(PABA)(2),[35] Sulphonamide being a structural analog to PABA opposes the growth of bacteria by

preventing folic acid (folate) synthesis, a compound needed by every cell for the biosynthesis of RNA, DNA and proteins. The sulphonamides(3) inhibit folic acid synthesis in susceptible bacteria by competitively blocking para-aminobenzoic acid (PABA).[36]
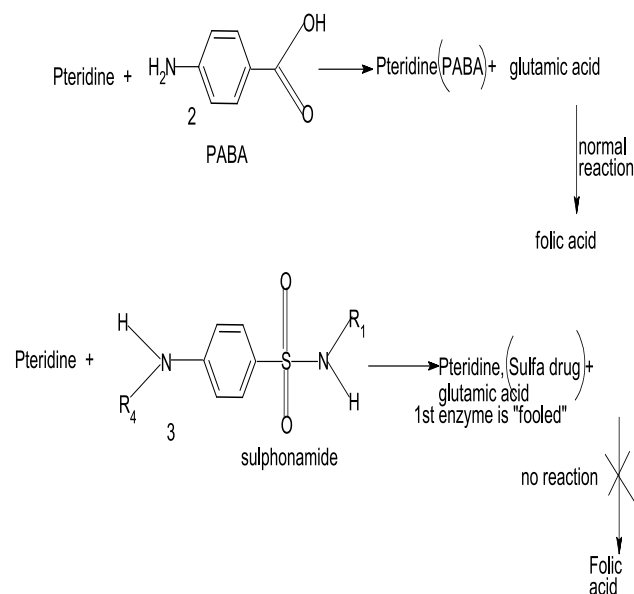
Scheme 1: Sulphonamide drug action pattern.

Application Of Sulphonamides As Antimicrobial Agents

The application of sulphonamides as antimicrobial agents dwindled in 1980s, yet sulphonamides are still a drug of choice for the treatment of acute and mild urinary and eye infections in many countries of the world.[37] In the recent times, sulfa-drugs such as sulfamethoxazole, sulfisoxazole, sulfadiazine and acetylsulfafurazole became drugs of greatest use.[38] Sulphonamides are the commonest veterinary antibiotics in china, and Europe because of the low cost.[39-40]

Resistance Mechanism of *Pseudomonas Aeruginosa*

The recalcitrance of *Pseudomonas aeruginosa* stems from the fact that this microorganism utilizes its advanced levels of intrinsic and acquired resistance mechanism against antibiotics. Additionally, *Pseudomena aeroginosa* was recently discovered to also employ an adaptive antibiotic resistance that involves biofilm- mediated resistance and multidrug resistant persister cells in relapse of infection.[41] The intrinsic antibiotic resistance of *Pseudomonas aeroginosa* is defined as its natural ability to neutralize or minimize the efficacy of antimicrobial agents *via* structural and functional characteristics,[42] and *Pseudomonas aeroginosa* accomplishes such through advanced mechanisms such as restriction of membrane permeability, efflux system strategy and generation of enzymes that are antibiotic-inactivating especially B-lactamases.[43] Restriction of the outer membrane permeability which is highly exhibited by *Pseudomonas aeruginosa* prevents the penetration of antibiotics to intracellular targets.[44-45] This fact makes polymyxins

currently a lead antibiotic against *Pseudomonas aeruginosa* because of its ability to successfully bind to the lipopolysaccharides (LPS) on the outer membrane of the microorganism thereby increasing cell membrane permeability and antibiotic uptake.[46] Moreover, the antibiotic-inactivating enzymes production by *Pseudomonas aeruginosa* enables the gram-negative bacteria to break down or modify antibiotics as a resistance mechanism. For instance many chemical bonds such as amides and esters are easily broken by enzymic hydrolysis of B-lactamases and aminoglycoside-modifying enzymes.[47-48]

Other possible ways through which *Pseudomonas aeruginosa* can increase its acquired antibiotic resistance are by mutational changes and acquisition of resistance genes,[49-50] through which the bacteria reduces the antibiotic uptake, modifies the antibiotic targets, over expresses efflux pumps and enzymes causing antibiotic inactivation.[43,51-52]

Resistance of *Pseudomonas aeruginosa* to Sulfonamides

Sulphonamide drugs were once a first line choice of antibiotics but things are presently different. Sulphonamide drugs function on the principle of selective toxicity and the basis for such selectivity is an enzyme called dihydropteroate synthase (DHPS) in the folic acid pathway. Mammals unlike bacteria do not depend on the endogenous synthesis of folic acid and therefore do not possess DHPS but utilize a folate uptake system which is not found in prokaryotes.[53] Isolated mutants in the dhps(*folp*) gene were found to exhibit a compromise between sulphonamide resistance and the performance of DHPS enzyme. In *Pseudomonas aeruginosa*, the resistance against sulphonamide is mediated by foreign *folp* transfer.[53] It is pertinent to say that the clinical resistance in *Pseudomonas aeruginosa* is plasmid-borne prompted by genes encoding alternative drug-resistant variants found in the enzyme dihydropteroate synthase (DHPS). These genes especially *sul1* and *sul2* after being sequenced were found to have almost the same frequency among clinical isolate and the corresponding DHPS enzymes exhibited significant insensitivity to sulphonamide drugs while binding normally to the *p*-aminobenzoic acid (PABA) substrate irrespective of the fact that the substrate and inhibitor were structurally similar.[53] Generally, the recalcitrance of bacteria to sulphonamide is attributed to the permeability barrier, efflux pumps and presence of target enzymes that exhibit naturally insensitivity, regulation, mutational and recombinational changes, acquired resistance.[1] It is worthy to note that *Pseudomonas aeruginosa* exhibit major resistance to sulphonamides *via* the permeability barrier and efflux pumps mechanisms.[54-55] *Pseudomonas aeruginosa* was found to have about two multiple drug efflux systems expressed by the outer membrane proteins OprM and OprJ and these represents two genetically different multidrug efflux systems found in the recalcitrant

species.[56-57] Kohler *et al*,[58] reported that OprM and OprJ- over expressing strains were found to exhibit high resistance to sulphonamide while mex ABoprM efflux system plays the major role in the intrinsic resistance of *Pseudomonas aeruginosa* to sulphonamide drugs[58] However, Grey and Hamilton[59] tested miller reported that amongst all the sulphonamide drugs, tested, sulphasiazine showed the highest activity, followed by sulphamethoxazole while sulphadimidine had the least activity against pseudomonas aeruginosa.[59]

Special Observation

It has been reported that certain amino acids can trigger biofilm disassembly in *Pseudomonas aeruginosa*[60] probably by increasing swimming motility.[61] Antimicrobial peptides which are employed against broad spectrum of pathogenic bacteria are known to contain about 12-50 amino acid residues.[62] It also has been observed that amino acid based sulphonamide derivatives exhibit excellent antimicrobial activities against *Pseudomonas aeruginosa*.[63-64] because amino acids have been found to potentiate the antimicrobial activities of sulphonamides.[65]

For instance, Eze et al[66] reported that a sulphonamide derivative 2-(4- methylbenzenesulfonamido) -4-(methylsulfanyl)-*N*-propylbutanamide synthesized from methionine an essential amino acid exhibited a significant inhibitory activity (MIC 6.45 mg/ml) against *Pseudomonas aeruginosa*. This compound was found to exhibit better minimum inhibitory concentration than commercially available ciprofloxacin (MIC, 9.05 mg/ml).[66] They also reported that *N*-Butyl-1-[(4-methylphenyl)sulphonyl]pyrolidine-2-carboxamide (MIC, 6.67 mg/ml) a proline derivative of sulphonamide displayed an improved antimicrobial potency against *Pseudomonas aeruginosa*. The minimum inhibitory concentration of the compound was also better than ciprofloxacin (MIC, 9.05 mg/ml).[67]

Egbujor and Okoro reported.[68] that some methionine based sulphonamide derivatives such as 2-{[(4-methylphenyl)sulphonyl])amino}-4-(methylsulphanyl)butanoic acid and 2-{acetyl[(4-methylphenyl)sulfonyl]amino} -4-(methylsulfanyl)butanoic acid had excellent antimicrobial effect on *Pseudomonas aeruginosa* with miniumum inhibitory concentrations as low as 0.7mg /ml and 0.8mg/ml respectively.[68] In a similar development, they reported that a serine-based sulphonamide derivatives 2-{acetyl[(4-methylphenyl) sulphonyl]amino}-3- hydroxyl propanamide had minimum inhibitory concentration (MIC 0.7 mg/ml) against pseudomonas aeruginosa.[69]

CONCLUSION

The clinical importance of sulphonamide drugs is gradually dwindling due to the wild spread of resistance to these inexpensive and effective drugs by bacteria especially *Pseudomonas aerugi*nosa and this has

necessitated the development of newer but expensive antibiotics. Obviously, *Pseudomonas aeruginosa* is uniquely problematic because of its complex mechanisms of drug resistance but observations have shown that the incorporation of certain bioactive amino acids into sulphonamide drug scaffold potentiated their antimicrobial activities against *Pseudomonas aeruginosa.* In view of promising improved potency against *Pseudomonas aeruginosa,* sulphonamides therefore need structural modifications that allow the incorporation of bioactive amino acids.

REFERENCES

1. Huovinen P. Resiatance to trimethoprim-sulfamethoxazole. *Clinical Infectious Diseases*, 2001; 32; 1608-14.
2. Apaydin S., Torok, M. Sulfonamide derivatives as multi-target agents for complex diseases. *Boorg Med Chem Letts*., 2019; 29(6): 2042-2050.
3. Benedetti, PGD: Advances in drug research. *Volume 16*. Edited by:Testa B. Academic Press. London and New York, 1987; 227-279.
4. Mengelers MJ, Hougee PE, Jansson LH, Van Miert AS: Structureactivity relationship between antibacterial activities and physicochemical properties of sulfonamides. *J Vet Pharmacol Therap,* 1997; 20: 276-283.
5. Cadena M, Durso LM, Miller DN, Wortmann C. Tetracycline and sulfonamide antibiotics resistance genes in soils from Nebraska organic farming operations. *Front. Microbial*., 2018; 28 June 2018. Doi:10.3389/fmicb.2018.01283.
6. Reeves DS, Bint AJ, and Bullock DW. Use of antibiotics: sulphonamides, co-trimoxazole, and tetracyclines. *The British Medical Journal,* 1978; 2(6134): 410-413.
7. Gaded AK, Mahajanshetti CS, Nimbalkar A and Raichurkar A. "Synthesis and antibacterial activity of some 5-guanyl hydrazone/thiocyanato-6-arylimidazo (2,1,-b) -1,3,4- thiadiazole-2-sulfon amide derivatives" European Journal of Medicinal Chemistry, 2003; 35(9): 853-857.
8. Ahmed A, Channar PA, Saeed A. Synthesis of sulfonamide, amide and amine hybrid pharmacophore, an entry of new class of carbonic anhydrase II inhibitors and evaluation of chemoinformatics and binding analysis. *Bioorg Chem*, 2019; 86: 624-630.
9. El-sayed NS, EL-Bendary ER, Mel-Ashry S and El-Kerdawy MM. "Synthesis and antituma, activity of new sulfonamide derivatives of thiadiazolo(3,2-a) Pyrimidines," *European Journal of Medicinal Chemistry*, 2011; 46(9): 3714-3720.
10. Garcia-Galan MJ, Diaz-cruz MS and Bercelo D. "Identification and determination of metabolites and degradation products of sulphonamide antibiofics" Trends in Analytical Chemistry, 2008; 27(11): 1008-1022.
11. Gidden AC, Gamage SA, Kendall JD. Synthesis and biological evaluation of solubilized sulfonamide analogues of the phosphatidylinositol 3-kinase inhibitor ZSTK474. *Bioorg. Med Chem,* 2019; 27(8): 1529-1545.
12. Cynthia M, Kahn, Scott line The Merk vetinary manual 10th Edition: Merk and Co. Inc. white house station N: J. USA, 2010.
13. Reddy NS, Mallireddigari MR, Cosenza S et al. Synthesis of new coumarin 3-(N-aryl) sulfonamides and their anticancer activity," *Bioorganic and Medicinal chemistry letters*, 2004; 14(15): 4093-4097.
14. Colmer-Hamood JA, Dzvova N, Kruczek C, Hamood AN. Chapter six-*In vitro* analysis of *Pseudomonas aeruginosa* virulence using conditions that mimic the environment at specific infection sites. *Progress in Molecular Biology and Translational Science,* 2016; 142: 151-191.
15. Balcht A, Smith R. *Pseudomonas aeruginosa:* Infections and treatment. *Informa Health Care,* 1994; 83-84.
16. Todar K. Todar's online textbook of bacteriology.textbookofbacteriology.net (2004-06-04). Retrieved on 2011-10-09.
17. Blanc, D. S., Petignat, C., Janin, B., Bille, J. & Francioli, P. Frequency and molecular diversity of Pseudomonas aeruginosa upon admission and during hospitalization: a prospective epidemiologic study. Clin Microbiol Infect, 1998; 4: 242–247.
18. Pechere JC and Kohler T. Patterns and modes of b-lactamresistance in Pseudomonas aeruginosa. *Clin Microbiol Infect*, 1999; 5(1): 15–18.
19. McGowan JE, Jr. Resistance in nonfermenting gramnegative bacteria: multidrug resistance to the maximum. *Am J Infect Control*, 2006; 34: 29–37.
20. Walker TS, Bais HP, Deziel E, Scweizer HP, Rahme LG, Fall R, Vivanco JM. *Pseudomonas aeruginosa*-plant root interactions. Pathogenicity, biofilm formation, and root exudation. Plant Physiology. 2004; 134(1): 320-31.
21. Rahme LG, Stevens EJ, Wolfort SF, Shao J, Tompkins RG, Ausubel FM. "Common virulence factors for bacterial pathogenicity in plants and animals". *Science*. 1995; 268(5219): 1899–902.
22. Rahme LG, Tan MW, Le L, Wong SM, Tompkins RG, Calderwood SB, Ausubel FM "Use of model plant hosts to identify Pseudomonas aeruginosa virulence factors". *Proceedings of the National Academy of Sciences of the United States of America*, 1997; 94(24): 13245-50.
23. Mahajan-Miklos S, Tan MW, Rahme LG, Ausubel FM. "Molecular mechanisms of bacterial virulence elucidated using a Pseudomonas aeruginosa-Caenorhabditis elegans pathogenesis model". *Cell*, 1999; 96(1): 47–56.
24. Martínez C, Pons E, Prats G, León J. "Salicylic acid regulates flowering time and links defence responses and reproductive development". *The Plant Journal*, 2004; 37(2): 209–17.
25. D'Argenio DA, Gallagher LA, Berg CA, Manoil C. "Drosophila as a model host for Pseudomonas

aeruginosa infection". *Journal of Bacteriology*, 2001; 183(4): 1466–71.

26. Miyata S, Casey M, Frank DW, Ausubel FM, Drenkard E. "Use of the Galleria mellonella caterpillar as a model host to study the role of the type III secretion system in Pseudomonas aeruginosa pathogenesis". *Infection and Immunity*, 2003; 71(5): 2404–13.

27. Karch, A.M. Focus On Nursing pharmacology (5th ed). Philadelphia: wolters Kluwer Health, Lippincott Williams, 2011; 115.

28. Chatzinikolaou I, Abi-Said D, Bodey GP, et al. Recent experience with *Pseudomonas aeruginosa* bacteremia in patients with cancer: retrospective analysis of 245 episodes. *Arch Intern Med*, 2000; 160: 501–9.

29. Todeschini G, Franchini M, Tecchio C, et al. Improved prognosis of *Pseudomonas aeruginosa* bacteremia in 127 consecutive neutropenic patients with hematologic malignancies. *Int J Infect Dis.,* 1998; 3: 99–104.

30. Vidal F, Mensa J, Almela M, et al. Epidemiology and outcome of *Pseudomonas aeruginosa* bacteremia, with special emphasis on the influence of antimicrobial therapy: analysis of 189 episodes. *Arch Intern Med,* 1996; 156: 2121–6.

31. Koburger J. Sulfonamide synthesis. *Chemische Berichte*, 1903; 36(1): 3626-3634.

32. Forster MO, Kunz E. Studies in the camphene series: Isomeric hydrazoximes of camphorquinone. *Chemical Society*, 1914; 105(1): 1732.

33. Zweifel GS, Nantz MH, and Somfai P. Mordern Oragnic Synthesis- An introduction. 1st Edition. W.H. Freeman, 2007; 1-30.

34. Laudadio G, Barmpoutsis E, Schotten C, Struik L, Govaerts, Browne DL and Noel T. Sulphonamide synthesis through electrochemical oxidative coupling of amines and thiols. *Journal of American Chemical Society,* 2019; 141(14): 5664-5668.

35. Lehne RA. Pharmacology for Nursing care (2nd edition). Philadelphia: W.B. Saunders company, 1994.

36. Katzung BG. Basic & clinical pharmacology (9th edition) united states of America: The McGraw-Hill companies, 2004.

37. Keys TF. Antimicrobials commonly used for tracctinfection: sulfonamides, trimethoprim-sufamethoxazole, nitrofurantoin, nalidixic acid. *Mayo Clin Proc,* 1977; 52(11): 680-2.

38. Albala DM, Prien EL,Jr, Gala HA. Urolithiasis as a hazard of sulfonamide therapy. *J Endourol,* 1994; 8(6): 401-3.

39. Ungemach F. Figures on quantities of antibacterials used for different purposes in the EU countries and interpretation. *Acta Vet Scand*, 1999; 93: 89–97. discussion 97–88, 111–117.

40. Kools SA, Moltmann JF, Knacker T. Estimating the use of veterinary medicines in the European Union. *Regul Toxicol Pharm,* 2008; 50: 59–65.

41. Pang Z, Raudonis R, Glick BR, Lin T, Cheng Z, Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and alternative therapeutic strategies. *Biotechnology Advances* 2019; 37: 177–192.

42. Blair JM, Webber MA., Baylay AJ, Ogbolu DO, Piddock LJ. Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol*, 2015; 13: 42–51.

43. Breidenstein EB, de la Fuente-Nunez C, Hancock RE. Pseudomonas aeruginosa: all roads lead to resistance. *Trends Microbiol*, 2011; 19: 419–426.

44. Bellido F, Martin NL, Siehnel RJ, Hancock RE. Reevaluation, using intact cells, of the exclusion limit and role of porin OprF in Pseudomonas aeruginosa outer membrane permeability. *J Bacteriol*, 1992; 174: 5196–5203.

45. Lambert PA. Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. *J R Soc Med*, 2002; 95(41): 22–26.

46. Zavascki AP, Goldani LZ, Li J, Nation RL. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. *J Antimicrob Chemother*, 2007; 60: 1206–1215.

47. Poole K. Aminoglycoside resistance in Pseudomonas aeruginosa. *Antimicrob Agents Chemother*, 2005; 49: 479–487.

48. Wolter DJ., Lister PD. Mechanisms of beta-lactam resistance among Pseudomonas aeruginosa. *Curr Pharm Des*, 2013; 19: 209–222.

49. Mandsberg LF, Ciofu O, Kirkby N, Christiansen LE, Poulsen HE, Hoiby N..Antibiotic resistance in Pseudomonas aeruginosa strains with increased mutationfrequency due to inactivation of the DNA oxidative repair system. *Antimicrob Agents Chemother*, 2009; 53: 2483–2491.

50. Welte W, Nestel U., Wacker T, Diederichs K. Structure and function of the porin channel. *Kidney Int,* 1995; 48: 930–940.

51. Hall RM., Collis CM. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. *Mol Microbiol*, 1995; 15: 593–600.

52. Odumosu BT., Adeniyi BA., Chandra R. Analysis of integrons and associated gene cassettes in clinical isolates of multidrug resistant Pseudomonas aeruginosafrom Southwest Nigeria. *Ann Clin Microbiol Antimicrob*, 2013; 12: 29.

53. Skold O. Sulfonamide resistance: mechanisms and trends. *Drug Resist Update,* 2000; 3(3): 155-160.

54. Kohler T, Kok M, Michea-Hamzehpour M, et al. Multidrug efflux in intrincid resistance to trimethoprim and sulfamethoxazole in *Pseudomonas aeruginosa. Antimicrob Agents Chemother,* 1996; 40: 2288–90.

55. Maseda H, Yoneyama H, Nakae T. Assignment of the substrate-selective subunits of the MexEI-OprN multidrug efflux pump of *Pseudomonas aeruginosa. Antimicrob Agents Chemother,* 2000; 44: 658–64.

56. Gibreel A, Sko¨ld O. Sulfonamide resistance in clinical isolates of *Campylobacter jejuni:* mutational changes in the chromosomal dihydropteroate

synthase. *Antimicrob Agents Chemother,* 1999; 43: 2156–60.

57. Akiba T, Koyama K, Ishiki Y, Kimura S, Fukushima T. On the mechanism of the development of multiple-drug–resistant clones of *Shigella.* Jpn. *J Microbiol,* 1960; 4: 219–27.

58. Kohler T, Epp SF, Curty LK and Pechere JC. Characterization of MexT, the regulator of the MexE-MexF-OprN multidrug efflux system of Pseudomonas aeruginosa. *J Bacteriol*, 1999; 181: 6300–6305.

59. Grey D and Hamilton-Miller. Sensitivity of Pseudomonas aeruginosa to sulphonamide and trimethoprim. *Journal of Medical Microbiology,* 1976; 10: 3.

60. Kolodkin-Gal I, Romero D, Cao S, Clardy J, Kolter R, Losick R. D-amino acids trigger biofilm disassembly. Science, 2010; 328: 627– 629.

61. Brandenburg KS, Rodriguez KJ, McAnulty JF, Murphy CJ, Abbott NL, Schurr MJ, Czuprynskia CJ. Tryptophan Inhibits Biofilm Formation by *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*, 2013; 57(4): 1921–1925.

62. Bjarnsholt T, Kirketerp-Møller Jensen PØ, K, Madsen KG, Phipps R, Krogfelt K, Høiby N, Givskov M. Why chronic wounds will not heal: a novel hypothesis. Wound Repair Regen, 2008; 16: 2–10.

63. Egbujor MC, Okoro UC, Okafor S. Design, synthesis, molecular docking, antimicrobial and antioxidant activities of new phenylsulfamoyl carboxylic acids of pharmacological interest. *Med Chem Res*., 2019; 28: 2118–2127. Doi:10.1007/s00044-019-02440-3.

64. Ugwu DI, Okoro UC and Chukwura TD. Nickel catalyzed synthesis of N-aryl and N-hetertoaryl substituted benzene sulphonamides and their biological activity evaluation. *Med. Chem,* 2014; 4: 357-360.

65. Egbujor MC, Okoro UC, Okafor S and Nwankwo N.E. Synthesis, characterization and *in silico* studies of novel alkanoylated 4-methylphenyl sulphonamoyl carboxylic acids as potential antimicrobial and antioxidantagents. *Inter. J. Pharm. Phytopharm. Res,* 2019; 9(3): 89-97.

66. Eze FU, Okoro UC, Ugwu DI, Okafor SN. New carboxamide bearing benzenesulphonamides: synthesis, molecular docking and pharmacological properties. *Biorganic Chemistry,* 2019; 92: 103265.

67. Eze FU, Okoro UC, Ugwu DI, Okafor SN. Biological activity evaluation of some new benzenesulphonamide derivatives. *Front. Chem*, 2019; 7: 634.

68. Egbujor, MC and Okoro UC New methionine-based *p*-toluenesulphonamoyl carboxamide derivatives as antimicrobial and antioxidant agents: Design, synthesis. *J. Pharm. Res. Int.,* 2019; 28(1): 1-12. Doi:10.9734/jpri/2019/v28i130192.

69. Egbujor MC, Okoro UC, Okafor S and Nwankwo NE. Design, Synthesis and Molecular Docking Of Novel Serine-Based Sulphonamide Bioactive Compounds as Potential Antioxidant and Antimicrobial Agents. *Indo American Journal of Pharmaceutical Science,* 2019; 06(06): 12232-12240. doi:10.5281/zenodo.3250306.