DOI: https://doi.org/10.53350/pjmhs22166810

ORIGINAL ARTICLE

Chiral Phthalimides Demonstrated Bactericidal Effect Against Multi Drug Resistant Hyper Virulent Klebsiella Pneumonia

ISRAR ALI KHAN[1], SALMAN AHMAD[2], FAHAD ULLAH[3], FALAK NIAZ[4], SAJID IQBAL[5], EJAZ ALI KHAN[6], MOMIN KHAN[7], SHER WALI KHAN[8]

[1,2,3,4]*Institute of Basic Medical Sciences, Khyber Medical University, Peshawar*
[5]*Department of Microbiology, Abdul Wali khan University, Mardan*
[6]*Pediatrics, TMO Kuwait Teaching Hospital, Peshawar*
[7]*Institute of Basic Medical Sciences, Khyber Medical University, Peshawar*
[8]*Department of Chemistry, Shaheed Benazir Bhutto University, Sheringal Dir Upper,1880 KP*
Corresponding author: Salman Ahmad, Email: salmanahmad.ibms.edu.pk

ABSTRACT

Bacterial resistance to antibiotics is global issue. Many antibiotics which is used is used in common practice against bacterial strain are no more effective against it. Same is the case of hyper virulent Klebsiella pneumoniae (hvKp) mostly clinical isolates of this bacteria are multi drug resistant and can cause a verity of infections. Our study aims to screen five novel chiral phthalimides (1, 3 dioxoisoindolin-2-y1)-4-methytopheonic acid labelled as FIB, 2-(1, 3-dioxoisoindlin-2y1)-4(methythio) butamic acid (FIC), 2-(1,3dioxoisoindolin-2yl)-3 meracaptopropanic acid (FIF) 2-(1-3-dioxoisoindolin-2yl)-3(4-hydroxyphnyl prophanic acid(FIH) and 3-(1, 3 dioxoisoindolin-2yl) prophanic acid (FII) against multi drug resistant hvKp and to check its cytotoxic effect against human red blood cells (RBC's). Activity of these compounds were measured through different techniques e.g. Agar well diffusion, disc diffusion and macro broth dilution method. Minimum inhibitory concentration (MIC) of these compounds were measured as well. All five compounds were bactericidal and were observed with different concentration of inhibition e.g. MIC for FIC, FIB, FII, FIF and FIH against hvKp were 6mg/ml, 25mg/ml, 25mg/ml, 50mg/ml and 100mg/ml. This result show that FIC have strong activity against hvKp as compared to other compounds. None of these five compounds were toxic to human RBC's. Chiral phthalimides showed strong antibacterial activity against hvKp and were none toxic to human RBC's.

INTRODUCTION

Klebsiella pneumoniae (Kp) belongs to Enterobacteriaceae family (1). This opportunistic bacterium causes a verity of infections e.g. bacteremia, pneumonia, diarrhea, urinary tract infections (UTI). Factors responsible for virulence in K. pneumoniae is lipopolysaccharides(2), capsule (3), siderophores (4,5), fimbriae (6,7), pore forming proteins (8), porins (9), efflux pump system septicemia, meningitis especially in immunocompromised individuals. On the basis of infections K. pneumoniae is classified to two types classical and hyper virulent Klebsiella pneumoniae(hvKp). In these two strains hvKp is considered as a mutated form of K.pneumoniae which is more harmful as compared to the classical one (10). In simple laboratory tests classical strain is differentiated from hyper virulent by a simple "string test" in which a wire loop is touched pneumonia to colony and picked, hyper virulent strain produce string upto 5mm or more (11). Genes responsible for hypermucoviscosity are RmpA and MagA (12). Classical K.pneumoniae is known for infections in immunocompromised individuals but hvKp can infect both healthy and immune compromised population (1,13). Hvkp cause infections from moderate to extreme level, it can cause pyogenic liver abscess in peoples which may not have any liver infection before exposing to it (14). This strain can also cause skin and delicate tissue infections such as cellulitis, necrotizing fasciitis, myositis and abscess in lungs, kidneys and neck area (15). Hvkp can cause community acquired (CAP) (16). Most of clinical isolates of K. pneumoniae obtained from different sites of infected of often resistant towards antibiotics used in normal practice against K. pneumoniae.

HvKp was first reported in Taiwan and southeast Asia in mid of 1980s and 1990s. from there it spread to rest of the world is now one of the leading cause of pyogenic liver abscess in Asia (17). In china hvKp constituted to 37.8 % of nosocomial infections (18),In Canada hvKp is reported in 8.2% of hospitalized patients (19).USA reported hvKp is involved in 6.3% of hospital acquired infections (20). Spain reported hvKp in 5.4% of hospitalized patients(21). South Korea and japan also reported hvKp from immunocompromised individuals (22). Italian study revealed that K.pneumoniae is resistant 85% of β-lactamase inhibitors, several 3rd generation antibiotics like Cephalosporin, 80% of fluoroquinolones, 70% of aminoglycosides and 65% of several folate pathway inhibitors (23). Infections caused either directly or involvement of hvKp are Spleen abscess (24–26) liver abscess (9), Bacterial peritonitis (17,27) community acquired pneumonia (16,17), complex para pneumonic infusions (28–30), urinary tract infection, bacteremia (27), lemierre syndrome (31), endophthalmitis (29,32), meningitis (33). As hvKp is becoming a super bug and causing infections globally and is resistant to most of commonly used antibiotics, same is the case in our country Pakistan. Some studies have reported on the resistance issue of hvKp, a study conducted in Islamabad (Pakistan) concluded that 25% of patients having kidney infections and UTI have hvKp infection, in which 33% isolates are multi drug resistant (34). Another study reported a strong increase in ESBL production and resistance in K. pneumoniae from 2002 to 2007 (35). A study carried out in Pakistan twenty different antibiotics were applied against Kp obtained from urine samples. percentage of results were observed as Amoxicillin have 0.1% of efficacy which is one of the common antibiotic used against Klebsiella, Doxycycline 11.5%, nitrofurantin15.5%, Amoxiclave 18.2%, Gentamicin 35.4% and so on (36). K. pneumoniae is now resistant towards Colistin which is considered as last line antibiotic against it (32). Another study conducted in 2020 in tertiary care units of Rawalpindi and Islamabad on clinical isolates of hvKp resulted in 36% over all resistance and 59% resistance to azetronam,55% to gentamicin,53% to ciprofloxacin, and 38% were resistant to carbapenemases (37). In Khyber Pakhtunkhwa (KPK) Pakistan to observe the resistance pattern of K. pneumoniae. A study conducted in Peshawar where clinical samples were taken which resulted in 71% resistance of Kp to 3 or more than three antibiotics in this study 18 antibiotics were applied (38).

Phthalimides are synthetic compounds with chemical formula $C_6H_4(CO)_2NH$. Addition of new sub group form novel phthalimides. they have gained great attention in the field of medicinal chemistry (39). Phthalimides have strong antibacterial activity against both gram positive and gram negative bacteria (40). Phthalimides binds to DNA grove and inhibit its replication (41).Combination of pyrazole 1, 2, 4-triazin, Imidazopyrazole, pyrazolepyrimidine with phthalimides targets DNA gyrase of bacteria (42). It also has antitumor activity as it targets α-TNF and inhibit inflammatory response (43,44). It has strong antifungal activity (45). Folpet is unit of phthalimides that inhibit conidial germination (46). Combination of benzathiazole and phthalic anhydride develop phthalimides which is proved to have strong anti-angiogenic properties (47). Keeping in view the anti-bacterial

potential of phthalimides this study was under taken to report the antibacterial activities of some novel phthalimides against hvKp.

METHODS AND MATERIALS

Study was carried out in Khyber medical university setup IBMS, microbiology laboratory.

Sample collection and culturing: Twenty different samples were collected from pus through sterile cotton swab, were brought to laboratory and cultured on macConkey agar for isolation of gram negative bacteria. Different colonies were obtained which was further sub cultured.

Identification of K. pneumonia: Identification of K. pneumoniae was done through different biochemical tests e.g. gram stain, catalase, oxidase, triple iron sugar test. For identifying hyper virulent strain, a well-known string test were performed.

Antibiotic susceptibility testing (AST): After isolation of HvKp for isolation of multi drug resistant strain Kirby disc diffusion method was performed. American type of culture collection (ATCC43816) for K. pneumoniae was used as a control in all experiments. Six different antibiotics were applied on isolated and identified strain e.g. Norfloxican 10µg (NOR), Erythromycin 15µg (E), ceftriaxone 30µg (CRO), amoxicillin+ clavulanic acid 30µg (AMC), Sulphamethaxazole + trimethophm 25µg (SXT), Nacdixic 30µg (NA).

Antibacterial activity of chiral phthalimides against hvKp: After isolation of MDR strain from hvKp five novel chiral phthalimides (1, 3 dioxoisoindolin-2-y1)-4-methytopheonic acid labelled as FIB, 2-(1, 3-dioxoisoindlin-2y1)-4(methythio) butamic acid (FIC), 2-(1,3dioxoisoindolin-2yl)-3 meracaptopropanic acid (FIF) 2-(1-3-dioxoisoindolin-2yl)-3(4-hydroxyphnyl prophanic acid(FIH) and 3-(1, 3 dioxoisoindolin-2yl) prophanic acid (FII) were applied on four different procedures e.g. Agar well diffusion, Disc diffusion method. Minimum inhibitory concentration of 5 different chiral phthalimides were measured with two different techniques e.g. Macro broth dilution and 96 wells plates method. Minimum bactericidal effect of these compounds were measured as well.

Agar well diffusion: Lawn was prepared through sterile cotton swab from bacterial solution compared with 0.5 McFarland standard inside biosafety cabinet. Wells were prepared on Muller Hinton agar (MHA) media through cork borer and different concentration of testing compounds were applied with same volume. Then plates were incubated for 24hrs on 37ºC.

Discs diffusion method: Lawn was prepared on MHA plates from hvKp inside biosafety cabinet in sterile condition. Blank disc cartages are available commercially which can absorb 20µl of liquid test compounds, after adding test compounds to discs it is incubated for 30 minutes to become dry. Then it is applied on plates containing prepared lawn and properly labeled and incubated for 24hrs on 37ºC.

Macro broth dilution: Minimum inhibitory concentration was measured against hvKp through macro broth dilution method in which sterile test tubes were used 3ml of Luria-bertani (LB) broth medium was poured to each tube in biosafety cabinet. Procedure was performed according to CLSI guide lines. Four different volumes (80, 60, 50 and 20µl) and four different concentrations (100,50,25,12mg/ml) was used. 24hrs fresh culture was taken and a standardized bacterial suspension of 0.5 McFarland's turbidity was added to each tube containing different volume and concentration of test compounds. Positive control (antibiotic free medium) and negative control (medium with antibiotic) was added. Tubes were properly covered with aluminum foil and were incubated for 24hrs at 37˚C.This experiment was performed in triplicate to result average of it.

Micro broth dilution: In this assay 96 wells plate was used.24 hrs fresh culture was used and 100µl of LB broth and 100µl of test compounds with different concentrations e.g. (200,100,50,25,12 and 6mg/ml) were added to each well. Culture suspension was compared to 0.5 McFarland standard. Positive control was well with bacterial suspension and L-B broth and negative was well with antibiotic disc. After this plates were kept in shaking incubator at 73ºC for 24 hrs.

Minimum bactericidal concentration: This experiment is performed for novel drugs, compounds or extracts, mostly after performing MIC to find out either these compounds are bacteriostatic or bactericidal. After observing inhibition in 96 wells plate after 24hrs, plates were opened in biosafety cabinet to assure aseptic conditions. A sterile disposable wire loop was dipped in each well and was streaked on macConkey agar which is ideal media for hvKp (gram negative). Same procedure was followed for wells of positive and negative controls. After culturing plates were incubated on 37ºC for 24 hrs.

Cytotoxicity: This is in vitro technique which is performed for every novel drug, compound or extract before it is introduced in vivo. In this experiment compounds were applied to human RBC's to check either it is toxic for human cells or not. Fresh blood was taken from healthy human (voluntarily) and was added to EDTA tubes to avoid clotting. Centrifugation was done on 3000rpm for 3 minutes to get RBC's. After centrifugation supernatants (plasma) was discarded, normal saline was added to tube and centrifuged again to get clear RBC's this step is performed 2-3 times. After 2-3 times of washing clear RBC's are obtained from which 200µl was added to a sterile tube containing 9.8ml of normal saline which makes 10ml solution. This solution is mixed and 100µl from prepared solution and 100µl of test compounds were added to sterile epidroff tube which makes 200µl solution. Positive control (0.5% triton X) and negative control as (saline) was added as well. Then the tubes are incubated for 1 hour in 37˚C. After incubation epidroff tubes containing solution were centrifuged on 10,000 rpm for 10 minutes and then its supernatants were collected and dispensed into cuvettes of spectrophotometer. Results can also be observed through Elisa reader or direct microscopy to observe ruptured cells.

RESULTS

Culturing: After sub culturing on nutrient agar pale mucoid colonies were obtained, on blood agar shinny white colonies were obtained and on macConkey agar pink mucoid colonies were obtained.

Identification: After gram stain microscopy pink rods were observed which is indication for K. pneumoniae. Triple sugar iron test results yellow butt yellow slant with no H_2S production and gas production which is strong indication of K pneumoniae. Further for catalase it resulted positive and negative for oxidase. String test results positive for our selected strains.

Antibiotic susceptibility test (Kirby disc diffusion method): In antibiotic sensitivity test our obtained strain was resistant to Norfloxican, ceftriaxone, amoxicillin +clavulanic acid, Sulphamethaxazole + trimethophm, Erythromycin and Nacdixic, while ATCC was sensitive to all as it was used as control.

Anti hvKp activity of chiral phthalimides

Agar well diffusion: All results were counted with diameter of well included. Zones were measured with scale. Table 1 represents numerical data of this experiment.

Table 1: Agar well diffusion,

Concentration	100mg/ml hvKp	ATCC	50mg/ml hvKp	ATCC
	Zone of inhibition (mm)			
FIH	21	23	20	21
FIB	20	21	16	17
FIC	17	19	15	16
FIF	16	17	11	13
FII	14	15	14	15

Significant activity (18mm) Good activity (16-18mm) Low activity (13-15mm) non-significant (9-12mm) No activity is below 9.

Disc diffusion method: Disc prepared from chiral phthalimides have no zones of inhibition it may be because of very less amount of absorption of compounds.

Minimum inhibitory concentration (MIC): For checking MIC of compounds or drug different techniques can be used we used 2 different techniques agar test-tube method and 24 wells plate method.

Macro broth dilution

Broth Test-tube method: In this experiment nutrient broth was used in same volume 3µl in each test tube. 4 different volumes e.g. 80,60,50 and 20 µl and 4 different concentrations e.g.100,50,25 and 12mg were used in this experiment table 2 show results of this experiment**.**

Table 2: Macro broth dilution

Volume	80µl		60µl		50µl		20µl	
Concentration	100mg/ml	50mg/ml	100mg/ml	50mg/ml	25mg/ml	12mg/ml	100mg/ml	50mg/ml
FII	-	-	-	-	-	+	+	+
FIC	-	-	-	-	-	+	+	+
FIH	-	-	+	+	+	+	+	+
FIF	-	-	-	-	-	+	+	+
FIB	-	+	+	+	+	+	+	+

Note; In this table negative (-) show inhibited growth while positive (+) show occurred growth.

Micro broth dilution method

96 wells plate method: In this technique sterile 96 wells plate was used and six different concentrations of compounds with same volume was used table 3 represents results of 96 well plate method.

Table 3: 24 wells plate method

Concentrations	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12 mg/ml	06 mg/ml
FII	-	-	-	-	+	+
FIC	-	-	-	+	+	+
FIH	-	+	+	+	+	+
FIF	-	-	-	+	+	+
FIB	-	-	-	-	+	+

Note; In this table negative (-) represent no growth while positive (+) represent growth. Control were observed with desired and accurate results.

Minimum bactericidal effect (MBC)L For conformation of our compounds that either it is bactericidal or bacteriostatic we performed MBC. All of our used compounds were bactericidal at different concentration mentioned in table 4.

Table 4: Minimum bactericidal effect.

Concentrations	200mg	100mg	50mg	25mg	12mg	06mg
FII	-	-	-	-	+	+
FIC	-	-	-	-	-	+
FIH	-	-	+	+	+	+
FIF	-	-	-	-	+	+
FIB	-	-	+	+	+	+

Note; In this table negative (-) represent no growth while positive (+) represent growth

This table show that 25mg/ml FII, 12mg/ml FIC, 100mg/ml FIH, 100mg/ml FIF, 100mg/ml FIB is bactericidal for hvKp.

Cytotoxicity: Cytotoxicity can be observed through different techniques e.g. spectrophotometer, Elisa reader and direct microscopy. In direct microscopy no ruptured RBC's were observed. Results of Spectrophotometer is mentioned in table 5.

Table 5: Cytotoxicity

Compounds tags 100-200µg/ml	Percentage of hemolysis	Remarks
FII	4%	Non toxic
FIH	3%	Non toxic
FIB	4%	Non toxic
FIC	2%	Non toxic
FIF	1%	Non toxic

More than 5% is considered as hemolytic,2-4% is considered slightly hemolytic, less than 2% is non hemolytic.

DISCUSSION

Bacterial resistance is increasing day by day which is a serious threat to health throughout the world. Bacterial strain adopts different techniques to become resistant e.g. efflux pump, ESBL production, capsule and many more. In our study we observed multi drug resistant hvKp strain which have strong correlation with liver abscess(48). K. pneumoniae is part of normal flora but is opportunistic pathogen and can cause a verity of infections (45,49,50). Phthalimides in combination with other subunits have many therapeutic activities like antibacterial, antifungal, antiparasitic, anti-inflammatory and many more. (95,83,91). In our study we screened five novel chiral phthalimides against MDR hvKp to evaluate its antibacterial and cytotoxic effect against human RBC's. Compounds were tagged according to formula e.g. FIC, FIB, FII, FIH, FIH. These compounds were applied through different techniques to check its antibacterial activity against hvKp e.g. Agar well diffusion, disc diffusion, macro broth dilution. American type of culture collection(ATCC43816) was used a control in each experiment. Chiral phthalimides resulted in strong antibacterial effect against MDR hvKp at varying concentration which supports study of Rua-B-aluman and H-Jelali that phthalimides have antibacterial activity (51,52). Minimum inhibitory concentration of these compounds were done through 96 wells plate method in which we find out MIC for FIC 6mg/ml, FIB 25mg/ml, FII 25mg/ml, FIF 50mg/ml and FIH 100mg/ml. A study conducted in Poland performed same technique for MIC(53). None of our compounds was toxic to RBC's in cytotoxicity experiment saline was used as positive and 0.5% triton X as positive control with accurate result (49).

REFERENCES

1. Paczosa MK, Mecsas J. Klebsiella pneumoniae: Going on the Offense with a Strong Defense. Microbiol Mol Biol Rev. 2016 Sep;80(3):629–61.
2. De Majumdar S, Yu J, Fookes M, McAteer SP, Llobet E, Finn S, et al. Elucidation of the RamA Regulon in Klebsiella pneumoniae Reveals a Role in LPS Regulation. Hauser A, editor. PLOS Pathog. 2015 Jan 29;11(1):e1004627.
3. Lawlor MS, Handley SA, Miller VL. Comparison of the Host Responses to Wild-Type and cpsB Mutant Klebsiella pneumoniae Infections. Infect Immun. 2006 Sep;74(9):5402–7.
4. Carniel E. The Yersinia high-pathogenicity island: an iron-uptake island. Microbes Infect. 2001 Jun;3(7):561–9.
5. Miethke M, Marahiel MA. Siderophore-Based Iron Acquisition and Pathogen Control. Microbiol Mol Biol Rev. 2007 Sep;71(3):413–51.
6. Xiao X, Yao B, Zhou Q, Zhang J. Microbiological characteristics of hypermucoviscous Klebsiella pneumoniae isolates from different body fluids. J Infect Dev Ctries. 2017 Sep 30;11(09):672–8.
7. Struve C, Bojer M, Krogfelt KA. Characterization of Klebsiella pneumoniae Type 1 Fimbriae by Detection of Phase Variation during Colonization and Infection and Impact on Virulence. Infect Immun. 2008 Sep;76(9):4055–65.
8. Hsieh P-F, Liu J-Y, Pan Y-J, Wu M-C, Lin T-L, Huang Y-T, et al. Klebsiella pneumoniae Peptidoglycan-Associated Lipoprotein and Murein Lipoprotein Contribute to Serum Resistance, Antiphagocytosis, and Proinflammatory Cytokine Stimulation. J Infect Dis. 2013 Nov 15;208(10):1580–9.
9. Tsai F-C, Huang Y-T, Chang L-Y, Wang J-T. Pyogenic Liver Abscess as Endemic Disease, Taiwan. Emerg Infect Dis. 2008 Oct;14(10):1592–600.
10. Choby JE, Howard-Anderson J, Weiss DS. Hypervirulent Klebsiella pneumoniae – clinical and molecular perspectives. J Intern Med. 2020 Mar;287(3):283–300.
11. Sánchez-López J. Hypermucoviscous Klebsiella pneumoniae: A challenge in community acquired infection. 2019;3.
12. Yeh K-M, Chang F-Y, Fung C-P, Lin J-C, Siu LK. magA is not a specific virulence gene for Klebsiella pneumoniae strains causing

liver abscess but is part of the capsular polysaccharide gene cluster of K. pneumoniae serotype K1. J Med Microbiol. 2006 Jun 1;55(6):803–4.
13. Liu Y-C, Cheng D-L, Lin C-L. Klebsiella pneumoniae Liver Abscess Associated With Septic Endophthalmitis. :4.
14. Kim HB, Wang M, Park CH, Kim E-C, Jacoby GA, Hooper DC. oqxAB Encoding a Multidrug Efflux Pump in Human Clinical Isolates of Enterobacteriaceae. Antimicrob Agents Chemother. 2009 Aug;53(8):3582–4.
15. Keller JJ. Risk of infections subsequent to pyogenic liver abscess: a nationwide population-based study. :6.
16. Ko W-C. Community-Acquired Klebsiella pneumoniae Bacteremia: Global Differences in Clinical Patterns. Emerg Infect Dis. 2002 Feb;8(2):160–6.
17. Yu W-L, Ko W-C, Cheng K-C, Lee H-C, Ke D-S, Lee C-C, et al. Association between rmpA and magA Genes and Clinical Syndromes Caused by Klebsiella pneumoniae in Taiwan. Clin Infect Dis. 2006 May 15;42(10):1351–8.
18. Lan P, Jiang Y, Zhou J, Yu Y. A global perspective on the convergence of hypervirulence and carbapenem resistance in Klebsiella pneumoniae. J Glob Antimicrob Resist. 2021 Jun;25:26–34.
19. Peirano G, Pitout JD, Laupland KB, Meatherall B, Gregson DB. Population-Based Surveillance for Hypermucoviscosity Klebsiella pneumoniae Causing Community-Acquired Bacteremia in Calgary, Alberta. Can J Infect Dis Med Microbiol. 2013;24(3):e61–4.
20. Chou A, Nuila RE, Franco LM, Stager CE, Atmar RL, Zechiedrich L. Prevalence of hypervirulent Klebsiella pneumoniae-associated genes rmpA and magA in two tertiary hospitals in Houston, TX, USA. J Med Microbiol. 2016 Sep 1;65(9):1047–8.
21. Cubero M, Grau I, Tubau F, Pallarés R, Dominguez MA, Liñares J, et al. Hypervirulent Klebsiella pneumoniae clones causing bacteraemia in adults in a teaching hospital in Barcelona, Spain (2007–2013). Clin Microbiol Infect. 2016 Feb;22(2):154–60.
22. Harada S, Doi Y. Hypervirulent Klebsiella pneumoniae: a Call for Consensus Definition and International Collaboration. Diekema DJ, editor. J Clin Microbiol [Internet]. 2018 Sep [cited 2021 Aug 29];56(9). Available from: https://journals.asm.org/doi/10.1128/JCM.00959-18
23. Vuotto C, Longo F, Pascolini C, Donelli G, Balice MP, Libori MF, et al. Biofilm formation and antibiotic resistance in Klebsiella pneumoniae urinary strains. J Appl Microbiol. 2017 Oct;123(4):1003–18.
24. Shon AS, Bajwa RPS, Russo TA. Hypervirulent (hypermucoviscous) Klebsiella pneumoniae: A new and dangerous breed. Virulence. 2013 Feb 15;4(2):107–18.
25. Chang W-N, Huang C-R, Lu C-H, Chien C-C. Adult Klebsiella pneumoniae Meningitis in Taiwan: An Overview. 2012;21(2):10.
26. Lee C-R, Lee JH, Park KS, Jeon JH, Kim YB, Cha C-J, et al. Antimicrobial Resistance of Hypervirulent Klebsiella pneumoniae: Epidemiology, Hypervirulence-Associated Determinants, and Resistance Mechanisms. Front Cell Infect Microbiol. 2017 Nov 21;7:483.
27. Lee H-C, Chuang Y-C, Yu W-L, Lee N-Y, Chang C-M, Ko N-Y, et al. Clinical implications of hypermucoviscosity phenotype in Klebsiella pneumoniae isolates: association with invasive syndrome in patients with community-acquired bacteraemia. J Intern Med. 2006 Jun;259(6):606–14.
28. Lin Y-T, Wang Y-P, Wang F-D, Fung C-P. Community-onset Klebsiella pneumoniae pneumonia in Taiwan: clinical features of the disease and associated microbiological characteristics of isolates from pneumonia and nasopharynx. Front Microbiol [Internet]. 2015 Feb 18 [cited 2021 Mar 21];9. Available from: http://journal.frontiersin.org/Article/10.3389/fmicb.2015.00122/abstract
29. Tu C-Y, Hsu W-H, Hsia T-C, Chen H-J, Chiu K-L, Hang L-W, et al. The changing pathogens of complicated parapneumonic effusions or empyemas in a medical intensive care unit. Intensive Care Med. 2006 Apr;32(4):570–6.
30. Tsang K, Leung W, Chan V, Lin A, Chu C. Complicated parapneumonic effusion and empyema thoracis: microbiology and predictors of adverse outcomes. :10.
31. Tsai S-S, Huang J-C, Chen S-T, Sun J-H, Wang C-C, Lin S-F, et al. Characteristics of Klebsiella pneumoniae Bacteremia in Community-acquired and Nosocomial Infections in Diabetic Patients. 2010;33(5):9.
32. Kidd TJ, Mills G, Sá-Pessoa J, Dumigan A, Frank CG, Insua JL, et al. A Klebsiella pneumoniae antibiotic resistance mechanism that subdues host defences and promotes virulence. EMBO Mol Med. 2017;18.
33. Decre D, Verdet C, Emirian A, Le Gourrierec T, Petit J-C, Offenstadt G, et al. Emerging Severe and Fatal Infections Due to Klebsiella pneumoniae in Two University Hospitals in France. J Clin Microbiol. 2011 Aug 1;49(8):3012–4.
34. Zafar S, Hanif S, Akhtar H, Faryal R. Emergence of hypervirulent K. pneumoniae causing complicated UTI in kidney stone patients. Microb Pathog. 2019 Oct;135:103647.
35. Khan E, Ejaz M, Zafar A, Jabeen K, Shakoor S, Inayat R, et al. Increased isolation of ESBL producing Klebsiella pneumoniae with emergence of carbapenem resistant isolates in Pakistan: Report from a tertiary care hospital. J Pak Med Assoc. 2010;60(3):5.
36. Abdullah FE, Mushtaq A, Irshad M, Rauf H, Afzal N, Rasheed A. Current efficacy of antibiotics against Klebsiella isolates from urine samples – A multi-centric experience in Karachi. Pak J Pharm Sci. 2013;6.
37. Imtiaz W, Syed Z, Rafaque Z, Andrews SC, Dasti JI. Analysis of Antibiotic Resistance and Virulence Traits (Genetic and Phenotypic) in Klebsiella pneumoniae Clinical Isolates from Pakistan: Identification of Significant Levels of Carbapenem and Colistin Resistance. Infect Drug Resist. 2021 Jan;Volume 14:227–36.
38. Ullah F, Malik SA, Ahmed J. Antimicrobial susceptibility pattern and ESBL prevalence in Klebsiella pneumoniae from urinary tract infections in the North-West of Pakistan. Afr J Microbiol Res. :5.
39. Fhid O, Zeglam TH, Saad SEA, Elmoug T, Eswayah A, Zitouni M, et al. Synthesis, characterization and pharmacological activity of some new phthalimide derivatives. 2014;6.
40. Pawar NS, Zalte SM. STUDIES AN ANTIMICROBIAL ACTIVITY OF VARIOUS N-SUBSTITUTED PHTHALIMIDES DERIVATIVES. 2014;2(4):7.
41. Al-Azzawi AM, Al-Razzak MSA. Synthesis, characterization and antimicrobial screening of new Schiff bases linked to phthalimidyl phenyl sulfonate moiety. Baghdad Sci J. 2014;11:438–46.
42. Othman IMM, Gad-Elkareem MAM, El-Naggar M, Nossier ES, Amr AE-GE. Novel phthalimide based analogues: design, synthesis, biological evaluation, and molecular docking studies. J Enzyme Inhib Med Chem. 2019 Jan 1;34(1):1259–70.
43. Sharma U, Kumar P, Kumar N, Singh B. Recent Advances in the Chemistry of Phthalimide Analogues and their Therapeutic Potential. Mini-Rev Med Chem. 2010 Jul 1;10(8):678–704.
44. Orzeszko A. Tumor necrosis factor-alpha production-regulating activity of phthalimide derivatives in genetically modified murine melanoma cells B78H1. Il Farm. 2003 May;58(5):371–6.
45. Imran M, Bisht AS, Asif M. A Review on Biological and Chemical Potential of Phthalimide and Maleimide Derivatives. Pharm Sci. 2019;17.
46. Masiello M, Somma S, Ghionna V, Logrieco AF, Moretti A. In Vitro and in Field Response of Different Fungicides against Aspergillus flavus and Fusarium Species Causing Ear Rot Disease of Maize. Toxins. 2019 Jan 1;11(1):11.
47. Mercurio A, Sharples L, Corbo F, Franchini C, Vacca A, Catalano A, et al. Phthalimide Derivative Shows Anti-angiogenic Activity in a 3D Microfluidic Model and No Teratogenicity in Zebrafish Embryos. Front Pharmacol. 2019 Apr 17;10:349.
48. Siu LK, Yeh K-M, Lin J-C, Fung C-P, Chang F-Y. Klebsiella pneumoniae liver abscess: a new invasive syndrome. Lancet Infect Dis. 2012 Nov;12(11):881–7.
49. Neun BW, Dobrovolskaia MA. Method for Analysis of Nanoparticle Hemolytic Properties In Vitro. In: McNeil SE, editor. Characterization of Nanoparticles Intended for Drug Delivery [Internet]. Totowa, NJ: Humana Press; 2011 [cited 2021 Mar 21]. p. 215–24. (Methods in Molecular Biology; vol. 697). Available from: http://link.springer.com/10.1007/978-1-60327-198-1_23
50. Lamie P, Philoppes J, El-Gendy A, Rarova L, Gruz J. Design, Synthesis and Evaluation of Novel Phthalimide Derivatives as in Vitro Anti-Microbial, Anti-Oxidant and Anti-Inflammatory Agents. Molecules. 2015 Sep 14;20(9):16620–42.
51. Haberecht HB, Nealon NJ, Gilliland JR, Holder AV, Runyan C, Oppel RC, et al. Antimicrobial-Resistant Escherichia coli from Environmental Waters in Northern Colorado. J Environ Public Health. 2019 Feb 18;2019:1–13.
52. Jelali H, Mansour L, Deniau E, Sauthier M, Hamdi N. An Efficient Synthesis of Phthalimides and Their Biological Activities. Polycycl Aromat Compd. 2020 Aug 20;1–8.
53. Orzeszko A, Gralewska R, Starościak BJ, Kazimierczuk Z. Synthesis and antimicrobial activity of new adamantane derivatives I. Acta Biochim Pol. 2000 Mar 31;47(1):87–94.