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Observing The Presence of Efflux Pump Activity in Some Multi Drug Resistant Clinically Isolated Bacterial Strains

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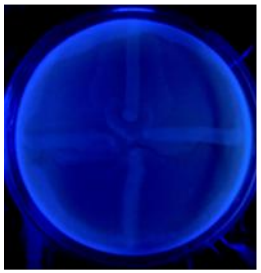
TSB Agar
+
Ethidium Bromide
(EtBr)

Pour into
a Petri dish

Inoculation

Incubation



No fluorescence
(EtBr is pumped by
efflux pumps)

Fluorescence
(EtBr cannot pumped
by efflux pumps)

Abstract:

Antibiotic resistance is one of the most common problems in antibacterial therapy. Several resistance mechanisms may lead to multi drug resistance (MDR), one of them is efflux pumps. Efflux pumps can be found in both prokaryotes and eukaryotes. With this mechanism, the entry of the drug into the cell is prevented or it is pumped out of the cell, thus the internal concentration of the drug is reduced and the drug cannot effect the cell. Due to this situation, which is frequently observed in microorganisms, antibiotic resistance occurs. If the efflux pump is inhibited, drugs may have a chance to affect cells. Some chemical inhibitors or natural compounds that provide this inhibition are commonly used for *in vitro* studies, but due to their toxicity problems, clinical use is not generally available. In this study, it was aimed to show the presence of efflux pump activity in some clinically isolated multi drug resistant strains, namely *Acinetobacter baumannii*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Klebsiella pneumoniae*, *Providencia rustigianii*, *Serratia odorifera*, *Shigella flexneri*, *Staphylococcus aureus* and *Streptococcus pneumoniae*. Ethidium Bromide (EtBr) was used to determine the presence of the efflux pump activity. A concentration range of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/L was used to observe the maximum EtBr concentration effluxed out the cell. After the incubation, the results were observed under UV light. As a result, it was observed that the maximum EtBr concentration effluxed out the cell changes between 1.0 and 2.0 mg/L for all strains used in the study.

Keywords: Antibiotic Resistance; Efflux Pumps; EtBr; MDR

Introduction

❖ One of the most common health problems of today is antibiotic resistance.

❖ One of the mechanisms that cause antibiotic resistance is efflux pumps.

❖ Efflux pumps can be found in both prokaryotes and eukaryotes.

❖ It first emerged in the 1980s as a result of the detection of *Escherichia coli* excretion of tetracyclines out of the cell (McMurry et.al., 1980; Özkanca, 2018; Altınöz, 2019).

Altınöz, E. (2019). Çoklu İlaç Direnci Gösteren Ve Efflux Pompa Sistemi Çalışan *Escherichia coli* Suşlarının Efflux Pompası İnhibitörlerine Karşı Cevaplarının Gözlenmesi. Yüksek Lisans Tezi. *Kastamonu Üniversitesi, Fen Bilimleri Enstitüsü*, Kastamonu.

McMurry, L., Petrucci, R. E., & Levy, S. B. (1980). Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*. *Proceedings of the national academy of sciences*, *77*(7), 3974-3977.

Özkanca, C. (2018). *Staphylococcus Aureus* ve *Escherichia Coli* Bakterilerinde Kademeli Olarak Oluşturulan Antibiyotik Direncinin Bitkisel Maddeler İle Engellenmesi. Yüksek Lisans Tezi. *İstanbul Üniversitesi*, *Sağlık Bilimleri Enstitüsü,* İstanbul.

❖ Until now, five most well-known efflux pump super families have been identified.

❖ ATP-Binding Cassette (ABC), Major Facilitator Superfamily (MFS), Multidrug And Toxin Extrusion (MATE), Small Multidrug Resistance (SMR), Resistance-Nodulation-Cell Division (RND).

❖ In addition to those, Proteobacterial Antimicrobial Compound Efflux (PACE) (Hassan et.al., 2013; Hassan et.al., 2015; Du et.al., 2018) and Drug Metabolite Transporter (DMT) (Putman et.al., 2000; Poole, 2004; Song and Wu, 2016) also defined recently.

Du, D., Wang-Kan, X., Neuberger, A., van Veen, H. W., Pos, K. M., Piddock, L. J., & Luisi, B. F. (2018). Multidrug efflux pumps: structure, function and regulation. *Nature Reviews Microbiology*, *16*(9), 523-539.

Hassan, K. A., Jackson, S. M., Penesyan, A., Patching, S. G., Tetu, S. G., Eijkelkamp, B. A., Brown, M. H., Henderson, P. J. F., & Paulsen, I. T. (2013). Transcriptomic and biochemical analyses identify a family of chlorhexidine efflux proteins. *Proceedings of the National Academy of Sciences*, *110*(50), 20254-20259.

Hassan, K. A., Liu, Q., Henderson, P. J., & Paulsen, I. T. (2015). Homologs of the *Acinetobacter baumannii* AceI transporter represent a new family of bacterial multidrug efflux systems. *MBio*, *6*(1), e01982-14.

Poole, K. (2004). Efflux-mediated multiresistance in Gram-negative bacteria. *Clinic al Microbiology and infection*, *10*(1), 12-26.

Putman, M., van Veen, H. W., & Konings, W. N. (2000). Molecular properties of bacterial multidrug transporters. *Microbiol. Mol. Biol. Rev.*, *64*(4), 672-693.

Song, L., & Wu, X. (2016). Development of efflux pump inhibitors in antituberculosis therapy. *International journal of antimicrobial agents*, *47*(6), 421-429.

❖ Schematized figure of 5 major super families are given in Figure 1 (Alav et.al., 2018).

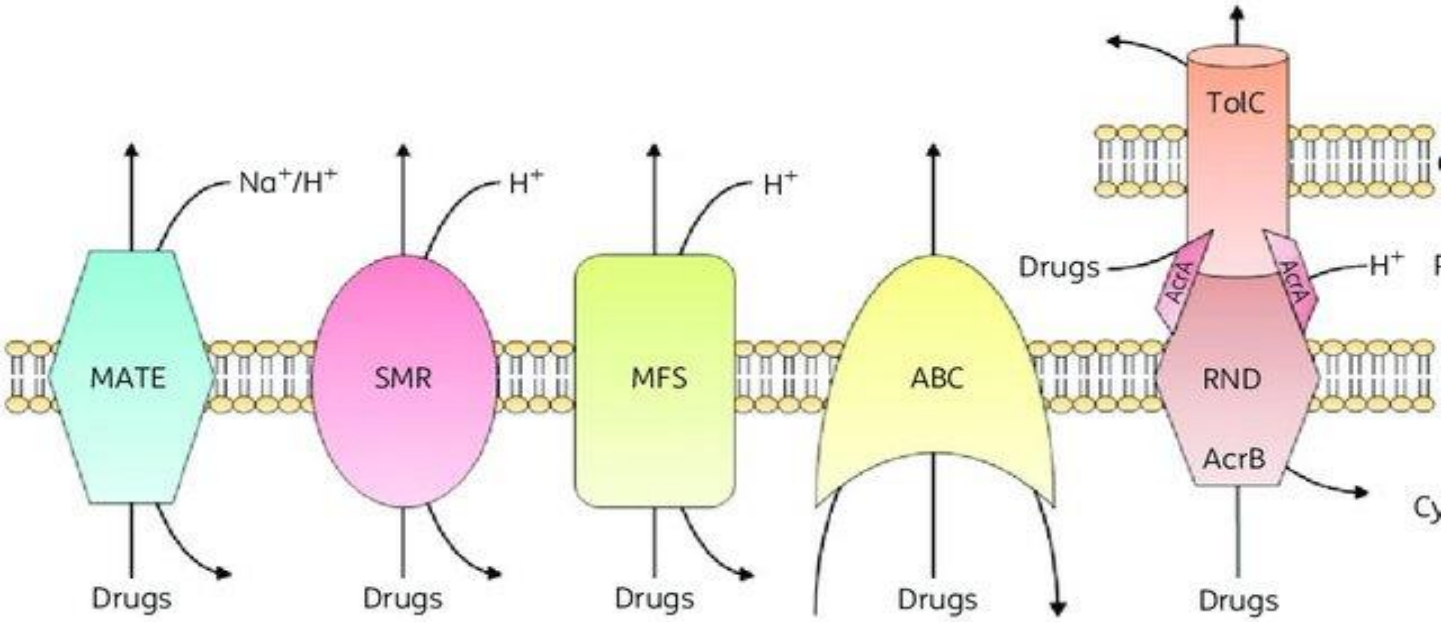


Figure 1

Alav, I., Sutton, J. M., & Rahman, K. M. (2018). Role of bacterial efflux pumps in biofilm formation. Journal of Antimicrobial Chemotherapy, 73(8), 2003-2020.

❖ Some known efflux pump chemical inhibitors:

➢ Verapamil (Bambeke et.al., 2006).

➢ Phenothiazine; chlorpromazine, piperidine and thioridazine (Amaral et.al., 1996; Viveiros and Amaral, 2001; Ordway et.al., 2003; Pule et.al., 2015).

➢2,4-dinitrophenol (DNP), Carbonyl Cyanide m-Chlorophenylhydrazine (CCCP) and valinomycin (Pasca et.al. 2005; Bambeke et.al., 2006; Ramón-García et.al., 2006; Pule vd. 2015).

➢ Phenylalanyl Arginine Beta Naphthylamide (PAβN) (Lomovskaya et.al., 2001).

Amaral, L., Kristiansen, J. E., Abebe, L. S., & Millett, W. (1996). Inhibition of the respiration of multi-drug resistant clinical isolates of *Mycobacterium tuberculosis* by thioridazine: potential use for initial therapy of freshly diagnosed tuberculosis. *Journal of Antimicrobial Chemotherapy*, *38*(6), 1049-1053.

Bambeke, V. F., Pagès, J. M. & Lee, V. J. (2006). Inhibitors of bacterial efflux pumps as adjuvants in antibiotic treatments and diagnostic tools for detection of resistance by efflux. *Recent patents on anti-infective drug discovery*, *1*(2), 157-175.

Lomovskaya, O., Warren, M. S., Lee, A., Galazzo, J., Fronko, R., Lee, M. A. Y., Blais, J., Cho, D., Chamberland, S., Renau, T., Leger, R., Hecker, S., Watkins, W., Hoshino, K., Ishida, H., & Lee, V. J. (2001). Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa*: novel agents for combination therapy. *Antimicrobial agents and chemotherapy*, *45*(1), 105-116. doi: 10.1128/AAC.45.1.105-116.2001.

Ordway, D., Viveiros, M., Leandro, C., Bettencourt, R., Almeida, J., Martins, M., Kristiansen, J. E., Molnar, J., & Amaral, L. (2003). Clinical concentrations of thioridazine kill intracellular multidrug-resistant *Mycobacterium tuberculosis*. *Antimicrobial agents and chemotherapy*, *47*(3), 917-922. doi: 10.1128/AAC.47.3.917–922.2003.

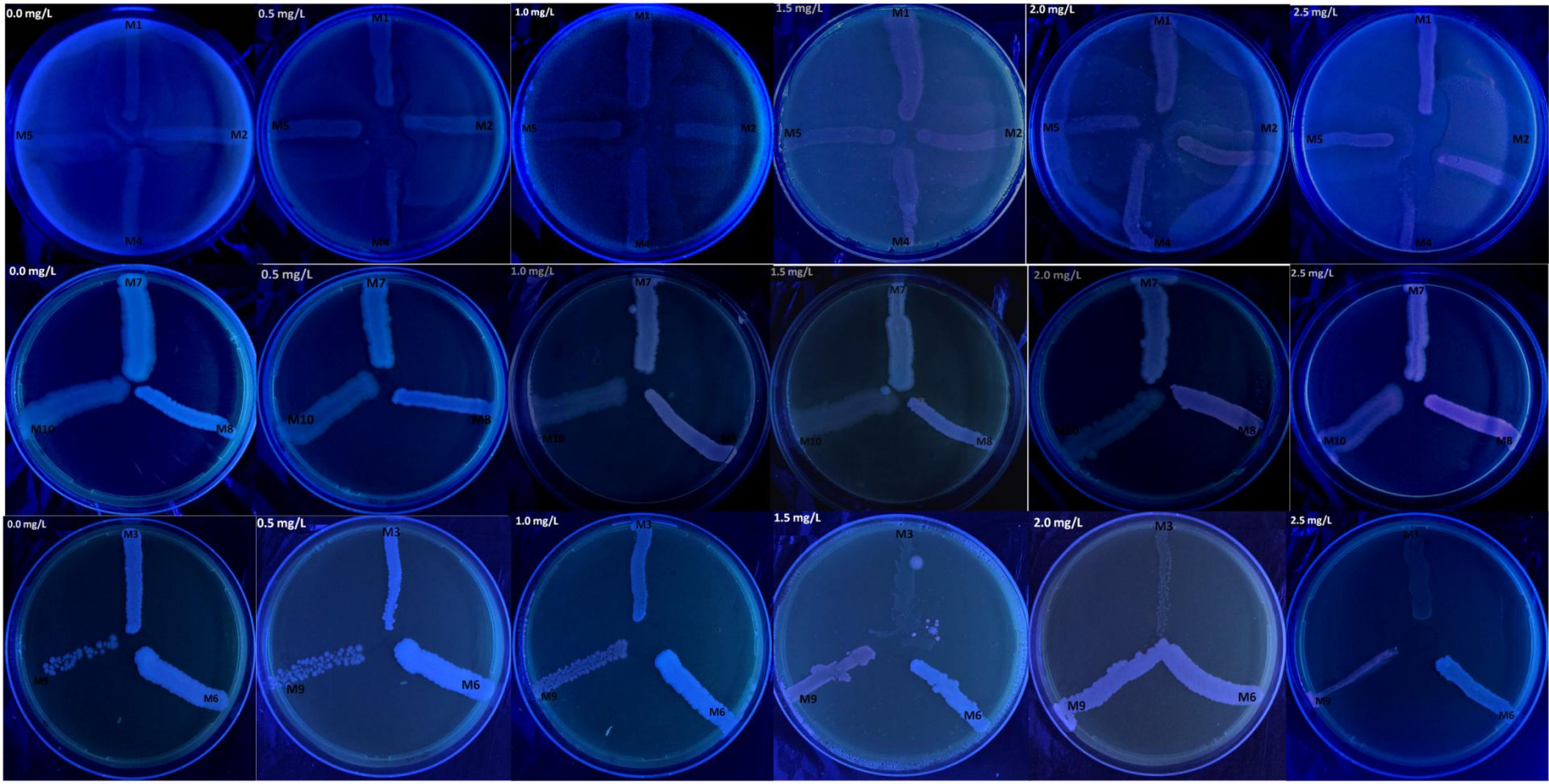
Pule, C. M., Sampson, S. L., Warren, R. M., Black, P. A., van Helden, P. D., Victor, T. C., & Louw, G. E. (2015). Efflux pump inhibitors: targeting mycobacterial efflux systems to enhance TB therapy. *Journal of Antimicrobial Chemotherapy*, *71*(1), 1-10. doi:10.1093/jac/dkv316.

Ramón-García, S., Martín, C., Aínsa, J. A., & De Rossi, E. (2006). Characterization of tetracycline resistance mediated by the efflux pump Tap from *Mycobacterium fortuitum*. *Journal of Antimicrobial Chemotherapy*, *57*(2), 252-259.

Viveiros, M., & Amaral, L. (2001). Enhancement of antibiotic activity against poly-drug resistant *Mycobacterium tuberculosis* by phenothiazines. *International journal of antimicrobial agents*, *17*(3), 225-228.

Results and discussion

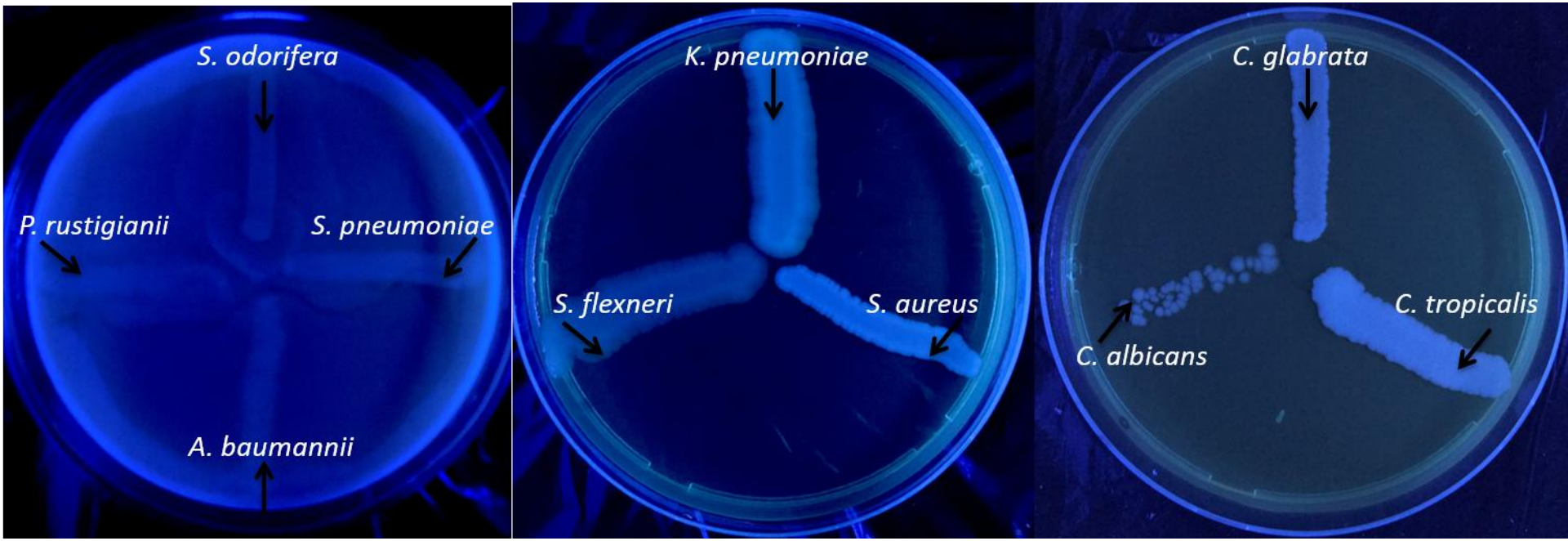
General view of efflux pump activity



0.0 mg/L 0.5 mg/L 1.0 mg/L 1.5 mg/L 2.0 mg/L 2.5 mg/L

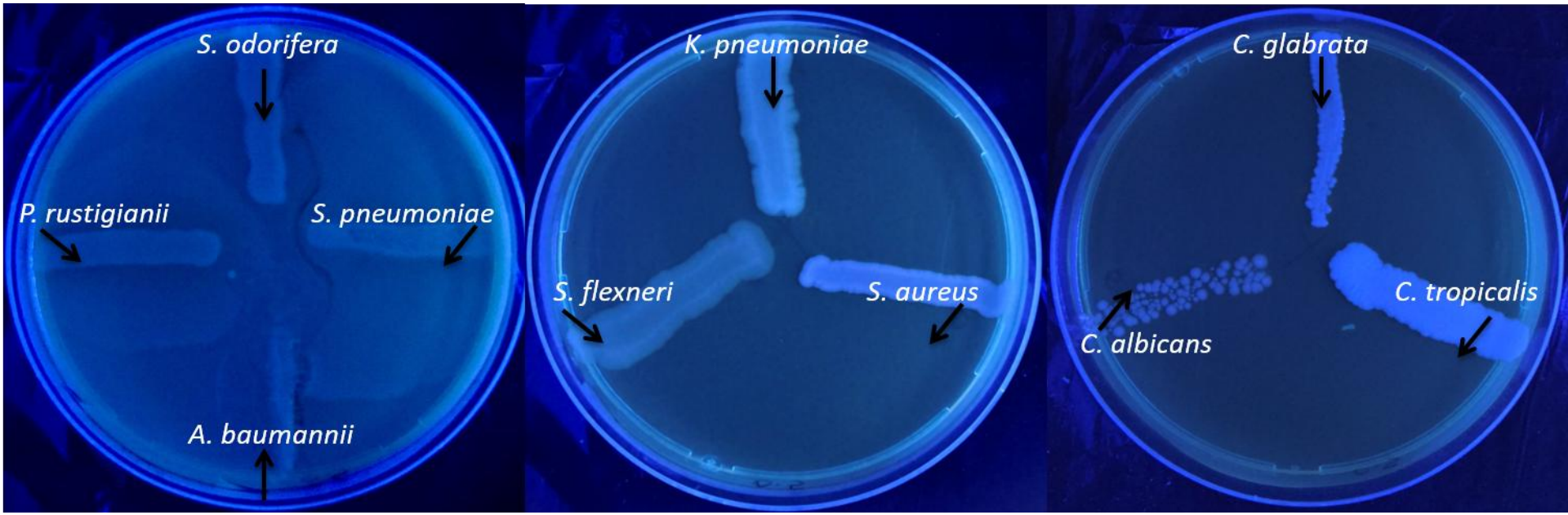
Figure 2

Results and discussion



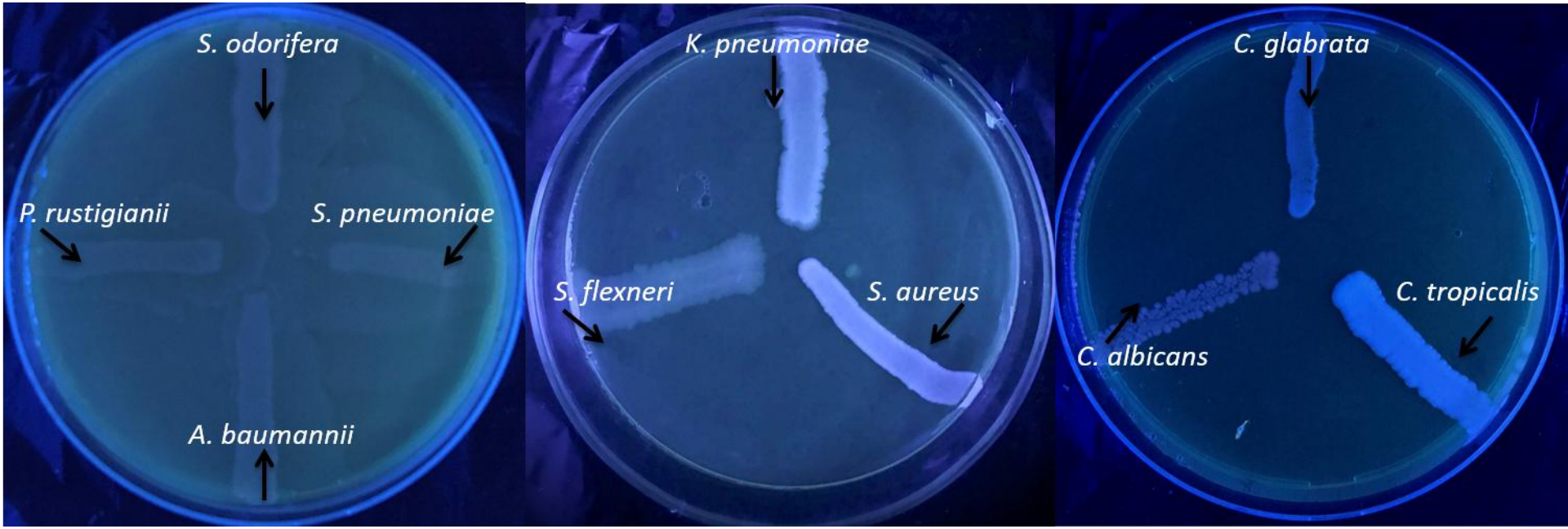
0.0 mg/L
No Ethidium Bromide (EtBr)
No fluorescence under UV

Results and discussion



0.5 mg/L
No fluorescence under UV
EtBr was effluxed in all microorgnisms

Results and discussion



1.0 mg/L

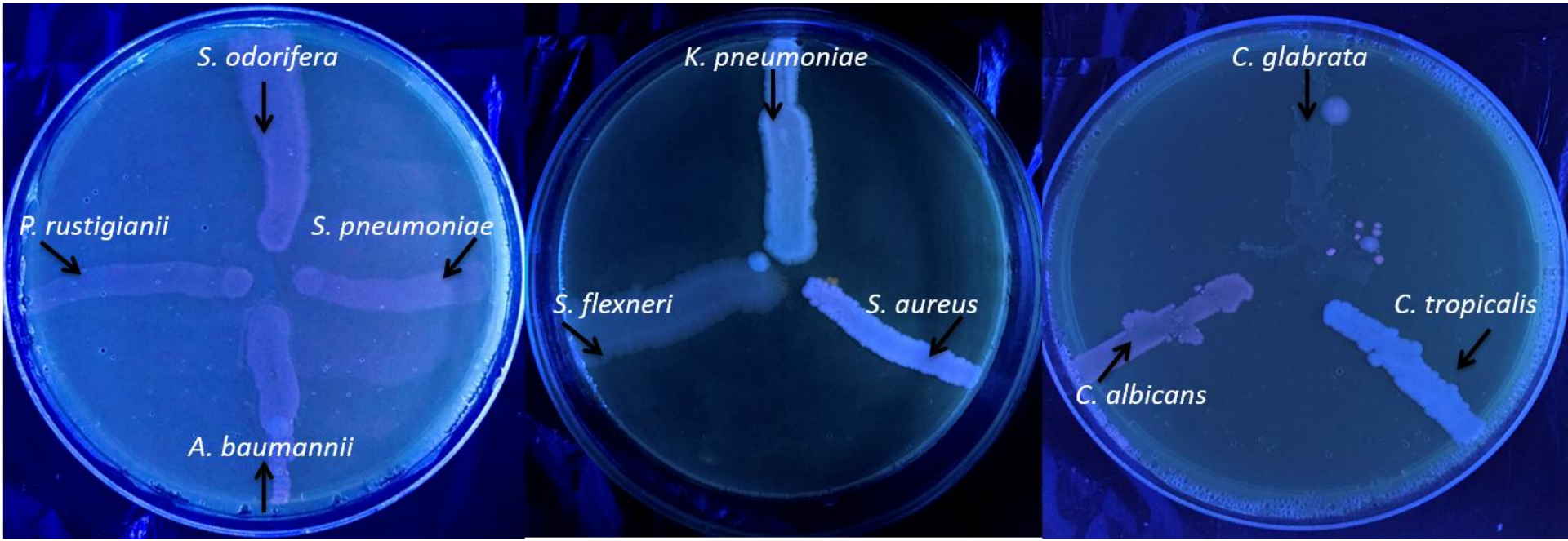
Fluorescence was observed under UV

Fluorescence ranking: *S. odorifera=S. pneumoniae=A. baumannii=P. rustigianii*

S. flexneri>K. pneumoniae=S. aureus

C. albicans>C. glabrata>C. tropicalis

Results and discussion



1.5 mg/L

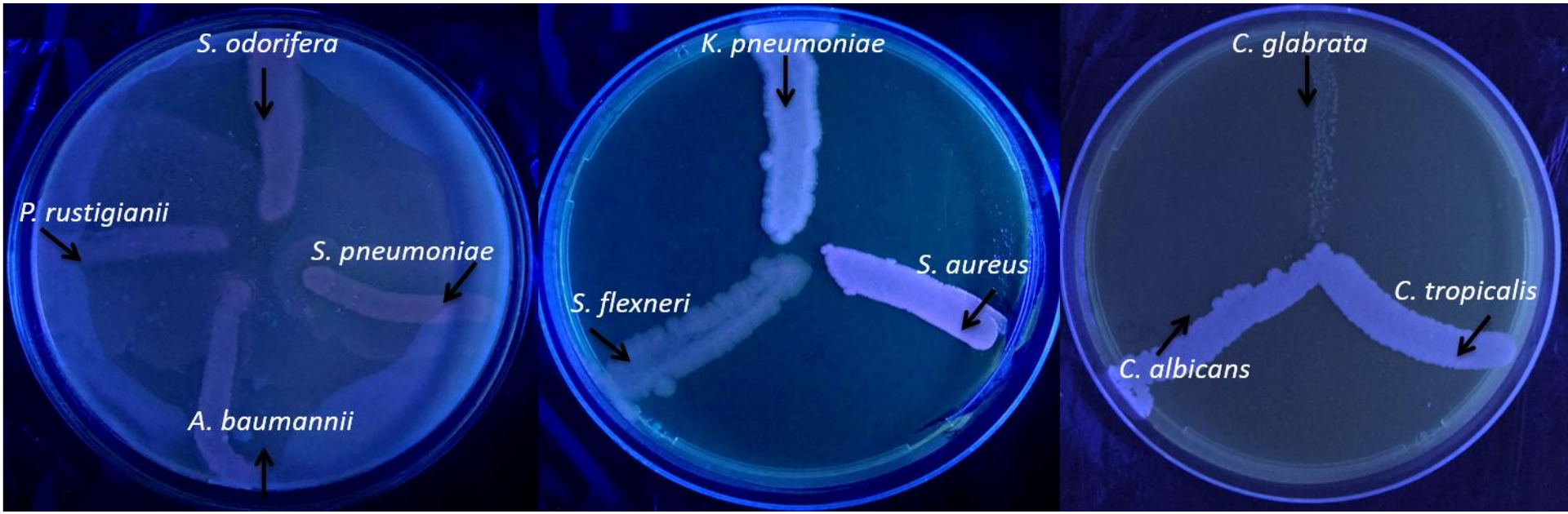
Fluorescence was observed under UV

Fluorescence ranking: *S. odorifera=S. pneumoniae=A. baumannii=P. Rustigianii*

S. flexneri>K. pneumoniae=S. aureus

C. albicans>C. glabrata>C. tropicalis

Results and discussion



2.0 mg/L

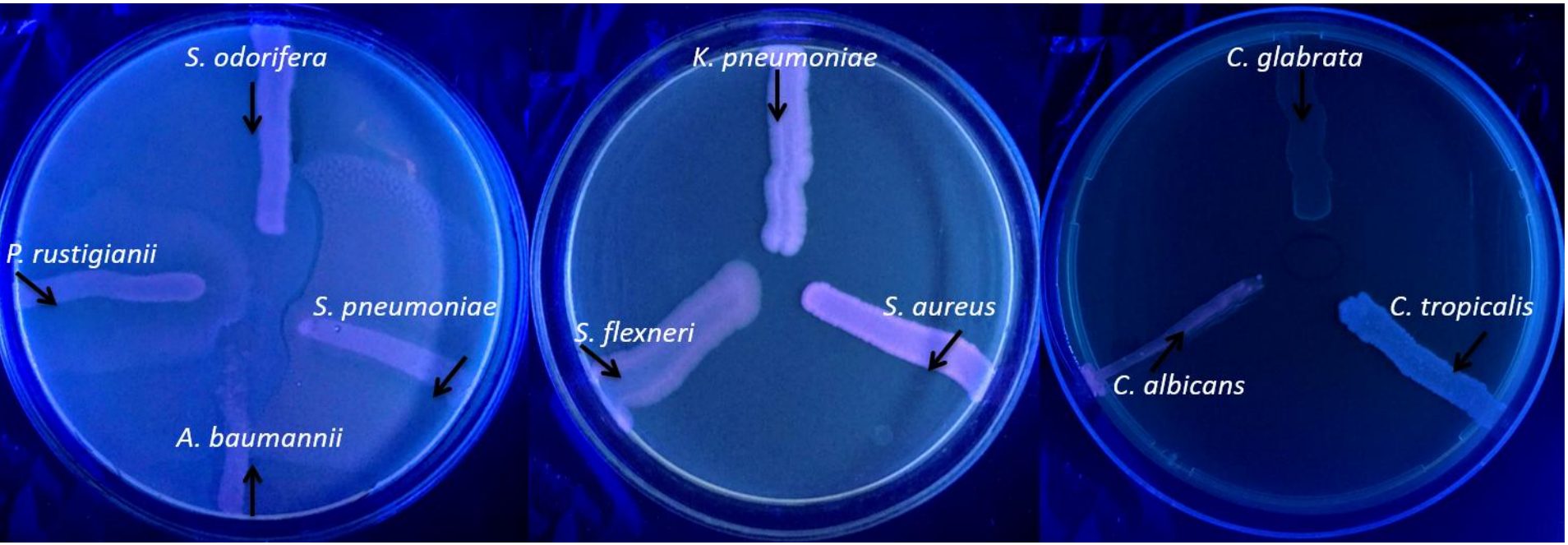
Fluorescence was observed under UV

Fluorescence ranking: *S. odorifera=S. pneumoniae=A. baumannii=P. rustigianii*

S. flexneri>S. aureus> K. pneumoniae

C. albicans=C. tropicalis> C. glabrata

Results and discussion



2.5 mg/L

Fluorescence was observed under UV

Fluorescence ranking: *S. odorifera=S. pneumoniae=A. baumannii=P. rustigianii*

S. flexneri>S. aureus> K. pneumoniae

C. albicans=C. tropicalis> C. glabrata

❖ 10 different clinical strains were used in the experiment. These strains are *Acinetobacter baumannii*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Klebsiella pneumoniae*, *Providencia rustigianii*, *Serratia odorifera*, *Shigella flexneri*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.

❖ EtBr dye was added to TSB agars and concentrations were prepared according to Martins et.al. (2010).

❖ It is normal to have different fluorescence rates under UV because microorganisms are different.

Martins, M., Couto, I., Viveiros, M., & Amaral, L. (2010). Identification of efflux-mediated multi-drug resistance in bacterial clinical isolates by two simple methods. In *Antibiotic resistance protocols* (pp. 143-157). Humana Press.

Conclusions

❖ As a result, the presence and activity of the efflux pump was determined in all 10 clinically isolated strains used.

❖ It was observed that the maximum EtBr concentration effluxed out the cell changes between 1.0 and 2.0 mg/L for all strains used in the study. The change was observed under UV.