Relationship Between the Quorum Network (Sensing/Quenching) and Clinical Features of Pneumonia and Bacteraemia Caused by A. baumannii

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Acinetobacter baumannii (Ab) is one of the most important pathogens associated with nosocomial infections, especially pneumonia. Interest in the Quorum network, i.e., Quorum Sensing (QS)/Quorum Quenching (QQ), in this pathogen has grown in recent years. The Quorum network plays an important role in regulating diverse virulence factors such as surface motility and bacterial competition through the type VI secretion system (T6SS), which is associated with bacterial invasiveness. In the present study, we investigated 30 clinical strains of A. baumannii isolated in the “II Spanish Study of A. baumannii GEIH-REIPI 2000-2010” (Genbank Umbrella Bioproject PRJNA422585), a multicentre study describing the relationship between the Quorum network in A. baumannii and the development of pneumonia and associated bacteraemia. Expression of the aidA gene (encoding the AidA protein, QQ enzyme) was lower (P < 0.001) in strains of A. baumannii isolated from patients with bacteraemic pneumonia than in strains isolated from patients with non-bacteraemic pneumonia. Moreover, aidA expression in the first type of strain was not regulated in the presence of environmental stress factors such as the 3-oxo-C12-HSL molecule (substrate of AidA protein, QQ activation) or H2O2 (inhibitor of AidA protein, QS activation). However, in the A. baumannii strains isolated from patients with non-bacteraemic pneumonia, aidA gene expression was regulated by stressors such as 3-oxo-C12-HSL and H2O2. In an in vivo Galleria mellonella model of A. baumannii infection, the A. baumannii ATCC 17978 strain was associated with higher mortality (100% at 24 h) than the mutant, abal-deficient, strain...
INTRODUCTION

Acinetobacter baumannii is a major cause of hospital-acquired infections associated with high mortality rates (Fuchs, 2016), usually affecting patients in Intensive Care Units (ICU) (del Mar Tomas et al., 2005; Lee et al., 2017). In these patients, A. baumannii causes infections such as pneumonia or, to a lesser extent, serious infections of the bloodstream (around 10% of clinical isolates of A. baumannii cause bacteraemia) (Cisneros and Rodríguez-Baño, 2002; El Kettani et al., 2017).

The success of this bacterium as a nosocomial pathogen, has been attributed to the following factors, amongst others: (i) high genetic versatility, facilitating rapid adaptation to stressful or unfavorable situations (Gayoso et al., 2014; Trastoy et al., 2018); (ii) ability to acquire new genes horizontally by the acquisition of plasmids and phages (López et al., 2018); (iii) ability to persist for a long time on animate and inanimate surfaces (resistance to desiccation) (Gayoso et al., 2014), which is generally attributed to biofilm formation; (iv) resistance to antimicrobial agents, including broad-spectrum antibiotics such as carbapenems, colistin, and tigecycline (Fernández-Cuenca et al., 2015), as well as to disinfectants and biocides (Fernández-García et al., 2018); and (v) high virulence (colonization, invasiveness, and cytotoxicity) (Rumbo et al., 2014; Wong et al., 2017). These characteristics contribute to the fact that nosocomial outbreaks caused by A. baumannii are difficult to control and that therapeutic options to treat infections are scarce or non-existent (Fernández-Cuenca et al., 2013). In February, 2017, the World Health Organization (WHO) published a list of “priority pathogens.” The list includes antibiotic resistant bacteria, considered a serious threat to human health and for which new antibiotics are urgently needed, and is headed by carbapenem-resistant A. baumannii (Tacconelli et al., 2018).

The Quorum Sensing (QS) network is generally used by Gram-negative bacterial pathogens to regulate biological processes such as virulence, conjugation, resistance, biofilm formation (which also depends on other factors such as the lytic enzymes responsible for peptidoglycan recycling: Vijayakumar et al., 2016), motility and bacterial competition, via secretion systems (T6SS), which are associated with greater invasiveness (LaSarre and Federle, 2013; López et al., 2017a,b). Two proteins (AbaI/AbaR) identified in A. baumannii have been described as homologs of the LuxI/LuxR system (by Quorum Quenching) (Gayoso et al., 2014; Trastoy et al., 2018). When a threshold concentration is reached, the AHL molecules present inside the cell are transported to its receptor (AbaR), putatively joining the lux-box, which is located 67 bp upstream of the ATG of AbaI, resulting in the synthesis of more AHL molecules (López et al., 2017b). The QS mechanism, on the other hand, acts naturally under environmental stress conditions such as the presence of bile salts in the gastrointestinal tract and H2O2 (ROS response) in the respiratory tract (López et al., 2017b).

A new enzyme (AidA) has recently been cloned in E. coli BL21 (DE3) and functionally characterized in clinical strains of A. baumannii capable of inhibiting their own QS (by Quorum Quenching) (López et al., 2017b). This enzyme acts by degrading signaling molecules such as N-(3-Oxododecanoyl), L-homoserine lactone (3-Oxo-C12-HSL), and N-dodecanoyl-L-homoserine lactone (C12-HSL), as confirmed by observation of inhibition of motility, biofilm formation and other virulence factors associated with activation of the Quorum Sensing system (López et al., 2017b). Other QQ enzymes have also recently been described in A. baumannii ATCC17978 (A1S_0383, A1S_2662, A1S_1876) (Mayer et al., 2018). Multiple QQ enzymes have been analyzed in diverse pathogens such as Pseudomonas aeruginosa (Zhang et al., 2011), Deinococcus radiodurans, Hyphomonas neptuniun, Photorhabdus luminicencens, and Rhizobium spp. (Kalai et al., 2011; Krysciak et al., 2011).

Based on these findings, in the present study, we examined the relationship between the global Quorum regulatory network (QS/QQ) mediated by the abaR (QS) and aidA (QQ) genes and the development of pneumonia and bacteraemia in clinical strains of A. baumannii isolated in the “II Spanish Study of A. baumannii GEIH-REIPI 2000-2010,” a multicentre study involving 45 Spanish hospitals and 246 patients. In addition, we used an in vivo infection model consisting of larvae of the wax moth Galleria mellonella to examine the relationship between the global QS/QQ and the development of mortality by a mutant abal (QS)-deficient strain of A. baumannii (A. baumannii ATCC17978Aabal) relative to that of the wild-type A. baumannii ATCC17978 strain.

MATERIALS AND METHODS

Bacteria and Samples

To carry out this study, we analyzed 30 clinical strains of A. baumannii from the 465 strains isolated in the “II Spanish Study of A. baumannii GEIH-REIPI 2000-2010” multicentre...
study (Genbank Umbrella Bioproject PRJNA422585). The multicentre study included 45 hospitals in Spain, in which new cases of colonization or infection by A. baumannii were analyzed between February and March 2010 (Villar et al., 2014). The 30 A. baumannii strains were all isolated from respiratory samples from patients with nosocomial pneumonia (n = 13: 6 with and 7 without bacteremia) or A. baumannii colonization of the lower respiratory tract (n = 17) (Sánchez-Encinales et al., 2017). Molecular typing was performed by Multilocus Sequence Typing (MLST) (Mosqueda et al., 2014). In addition, we used a killing assay with the Galleria mellonella infection model and an A. baumannii ATCC17978Δabal mutant strain (identified by Castañeda-Tamez et al., 2018).

The main clinical study variables included demographics, underlying diseases, mechanical ventilation, tracheostomy, colonization of lower respiratory airways, bacteremia pneumonia (Pn-B), non-bacteraemic pneumonia (Pn-NB) (Horan et al., 2008) and any cause of death during hospitalization.

To design the primers and probes of the QS genes and QQ enzymes, we analyzed the presence of QS genes (abaR and abaI) and the QQ enzyme (aida) in A. baumannii ATCC 17978 (Genbank genome accession numbers CP000521.1 [CP018664.1]) and in 100 A. baumannii genomes by consulting the “Integrated Microbial Genomes and Microbiomes” web page (https://img.jgi.doe.gov) and using nucleotide BLAST. The gene sequences in the search were selected from the Acinetobacter baumannii ATCC 17978 genome. A threshold of 1e-50 was used as the limit for analysis of the nucleotide sequence, where the e-value was defined as the probability of random alignments with the same score. We also calculated the percentage presence of these genes in the genomes (Figure S1).

**RNA Extraction**

RNA Extraction to Analyze the Quorum Regulatory Network (QS/QQ)

All clinical strains of A. baumannii were cultured on solid Luria-Bertani (LB) plates and incubated at 37°C for 24 h. One colony was removed and inoculated in liquid LB medium and incubated overnight at 37°C under stirring at 180 rpm. The inoculum was diluted (1:100) and allowed to grow until an optical density (OD600 nm) of 0.3 was reached. Aliquots of 10 µM of 3-Oxo-C12-HSL (QS-inactivating molecule by expression of the AidA protein) (Stacy et al., 2012; López et al., 2017b) and (10 µl) H2O2 were then added for 5 min (QS-activator by ROS response) (López et al., 2018). All controls were prepared by adding the same volumes of DMSO (dimethyl sulfoxide), 3-Oxo-C12-HSL and of sample, but with no H2O2. After incubation of the samples for 4 and 5 h in the presence of 3-Oxo-C12-HSL, to study the regulatory QS/QQ genes (abaR and aidA), as well as 5 min under H2O2 in static at 37°C, RNA was extracted using the High Pure RNA Isolation kit (Roche, Germany) and treated with Dnase. The extracted RNA was subsequently quantified as described above (Rumbo et al., 2013).

**RT-qPCR**

The studies were carried out with a Lightcycler 480 RNA MasterHydrosyis Probe (Roche, Germany), under the following conditions: reverse transcription at 63°C for 3 min, denaturation at 95°C for 30 s, followed by 45 cycles of 15 s at 95°C and 45 s at 60°C and, finally, cooling at 40°C for 30 s. The UPL primers and probes from conserved DNA regions identified by PCR (Universal Probe Library-Roche, Germany) used in the analysis are shown in Table 1.

All of the experiments were carried out in a final volume of 20 µl per well (18 µl of master mix and 2 µl of RNA). Each experiment was carried out in duplicate with two RNA extracts (50 ng/µl). For each strain, the expression of all genes, primers, and probes was normalized relative to the reference or housekeeping gene, rpoB, for RT-qPCR studies of Quorum sensing Primer sequences (5′-3′) with Taqman probes (Rumbo et al., 2013; López et al., 2017b). Analysis of the controls without reverse transcriptase confirmed the absence of DNA contamination.

**Galleria mellonella Infection Model**

The Galleria mellonella model was an adapted version of that developed by Peleg et al. (2009), Yang et al. (2015). The procedure was as follows: twelve G. mellonella larvae, acquired from TruLarvTM (BioSystems Technology, Exeter, Devon, UK), were each injected with 10 µl of a suspension of A. baumannii ATCC17978, or its isogenic deficient mutant A. baumannii ATCC17978Δabal, diluted in sterile phosphate buffer saline (PBS) and containing 8 × 10^4 CFU (± 0.5 log). The injection was performed with a Hamilton syringe (volume 100 µl) (Hamilton, Shanghai, China). In addition, a control group of twelve larvae were injected with 10 µl of sterile PBS. After being injected, the groups of larvae were placed in Petri dishes and incubated in darkness at 37°C. The number of dead larvae was recorded twice a day (morning and afternoon) for 6 days. The larvae were considered dead when they showed no movement in response to touch (Peleg et al., 2009).
Statistical Analysis
The gene expression studies were carried out in duplicate, and the data obtained were analyzed by Student’s t-test, implemented with GraphPad Prism v.6 software (GraphPad Software Inc. San Diego, CA). The graphs were constructed using the GraphPad program, and the results were represented as means and their respective standard deviations.

The mortality curves corresponding to the in vivo Galleria mellonella infection model were constructed using GraphPad Prism v.6 and the data were analyzed using the Log-rank test (Mantel-Cox). In both cases, p-values < 0.05 were considered statistically significant, and the data were expressed as mean values.

The statistical analyses were applied to the following categorical variables: age, sex, immunosuppressive treatment, surgery, ICU stay, mechanical ventilation, tracheostomy, severe sepsis, septic shock, and expression of the Quorum genes in A. baumannii. The results did not reveal any significant differences in the RE of the Quorum network genes (abaR, aidA) between clinical strains of A. baumannii isolated from colonized patients and strains of A. baumannii isolated from patients with pneumonia (0.086/0.094 vs. 0.071/0.095, p > 0.05).

We then proceeded to study the RE of the abaR and aidA genes in strains of A. baumannii from patients with pneumonia, differentiating the strains isolated from patients with bacteraemia (Pn-B) from those isolated from patients without bacteraemia (Pn-NB). The results did not reveal any significant differences in the RE of the Quorum network genes (abaR, aidA) between clinical strains of A. baumannii isolated from colonized patients and strains of A. baumannii isolated from patients with pneumonia (0.086/0.094 vs. 0.071/0.095, p > 0.05).

RESULTS
Study of the Gene Expression of the abaR and aidA Genes of the Quorum Network (QS/QQ)
The Relative Expression (RE) of the abaR and aidA genes of the Quorum network (QS/QQ) was quantified by RT-qPCR analysis of the 17 isolates of A. baumannii from colonized patients and of the 13 isolates of A. baumannii from patients with pneumonia (Figure 1A). The mean values (of two biological replicates) are presented in Tables 2, 3. These values were first used to determine any significant differences between the two types of strains in terms of gene expression in the Quorum network.

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Study of abaR/aidA Genes (QS/QQ) Under Stress Conditions (3-Oxo-C12-HSL and H2O2)
The values of the RE of the abaR and aidA genes (Quorum network) in the presence of 3-Oxo-C12-HSL (Inhibition of the QS) and H2O2 (Activation of the QS), obtained by RT-qPCR of the 13 isolates of A. baumannii from patients with pneumonia (differentiated from Pn-NB) are shown in Table 3, expressed as...
FIGURE 1 | (A) Relative Expression of the abaR and aidA genes in strains of A. baumannii from patients colonized with A. baumannii and patients with pneumonia caused by A. baumannii. No significant differences (p > 0.05) were detected in either case. (B) Relative expression of the abaR and aidA genes in isolates of A. baumannii from patients with bacteraemic and non-bacteraemic pneumonia. *p-value < 0.05 and ***p-value < 0.001.

TABLE 2 | Results of RT-qPCR analysis of the Relative Expression (RE) of the abaR and aidA genes (Quorum network genes) in the A. baumannii isolates from colonized patients.

| Strain (MLST<sup>a</sup>) | abaR (RE) | aidA (RE) |
|--------------------------|-----------|-----------|
| **STRAINS OF A. baumannii ISOLATED FROM COLONIZED PATIENTS** |           |           |
| Ab 22_GEIH-2010 (ST-52)  | 0.043     | 0.042     |
| Ab 38_GEIH-2010 (ST-2)   | 0.078     | 0.146     |
| Ab 59_GEIH-2010 (ST-269) | 0.081     | 0.003     |
| Ab 64_GEIH-2010 (ST-2)   | 0.076     | 0.213     |
| Ab 77_GEIH-2010 (ST-261) | 0.018     | 0.112     |
| Ab 112_GEIH-2010 (ST-263)| 0.112     | 0.038     |
| Ab 141_GEIH-2010 (ST-264)| **0.001** | 0.010     |
| Ab 177_GEIH-2010 (ST-2)  | **0.001** | 0.067     |
| Ab 205_GEIH-2010 (ST-2)  | 0.131     | 0.126     |
| Ab 288_GEIH-2010 (ST-263)| 0.067     | 0.199     |
| Ab 290_GEIH-2010 (ST-264)| 0.141     | 0.006     |
| Ab 294_GEIH-2010 (ST-2)  | 0.123     | 0.481     |
| Ab 326_GEIH-2010 (ST-2)  | 0.123     | 0.015     |
| Ab 354_GEIH-2010 (ST79)  | 0.052     | 0.020     |
| Ab 364_GEIH-2010 (ST-79) | **0.001** | 0.081     |
| Ab 399_GEIH-2010 (ST-79) | 0.061     | 0.050     |
| Ab 456_GEIH-2010 (ST-269)| 0.368     | 0.001     |

The results are expressed as the mean value of the two biological replicates. *MLST (Multilocus Sequence Typing by Pasteur database, https://pubmlst.org/) (Villar et al., 2014). In bold, RE ≤0.001 not detected by RT-qPCR.

In the clinical strains of A. baumannii isolated from Pn-NB (Figure 2), we observed regulation of expression of the aidA gene in the presence of 3-Oxo-C12-HSL (overexpression, RE ≥ 1.5) (Figure 2A) and of H₂O₂ [underexpression, RE ≤ 0.5 (Figure 2B)]. Expression of the abaR gene decreased significantly in the presence of the 3-Oxo-C12-HSL molecule (RE ≤ 0.5, Figure 2A).

In the clinical strains A. baumannii isolated from Pn-B (Figure 3), expression of the aidA gene was not regulated in the presence of 3-Oxo-C12-HSL or H₂O₂. However, the abaR gene was overexpressed in the presence of H₂O₂ (RE ≥ 1.5, Figure 3B).

These results indicate that the isolates of A. baumannii from Pn-NB may harbor a functional AidA protein (QQ enzyme), in contrast to the isolates of A. baumannii from Pn-B, which did not have this functional protein. Therefore, in the A. baumannii strains isolated from Pn-B, overexpression of the abaR gene (activation of the QS) in the presence of H₂O₂ (ROS response) would enable the development of the virulence factors favoring invasiveness, such as type VI secretion system (T6SS) and motility.

**Quorum Network (QS/QQ) Genes and Clinical Variables**

Analysis of the risk factors associated with the development of pneumonia vs. colonization by clinical strains of A. baumannii revealed only one statistically significant variable, i.e., diabetes mellitus (Table 4).

However, analysis of the risk factors associated with the development of bacteraemia in pneumonia caused by A. baumannii revealed underexpression of the aidA gene as the only statistically significant variable (p < 0.05) (Table 5).
Mortality in the *in vivo* *Galleria mellonella* Model

Injection of *G. mellonella* larvae with *A. baumannii* ATCC17978 at a concentration of 8 x 10⁴ CFU/larva (± 0.5 log) caused 100% mortality after 24 h, whereas injection of the larvae with the same concentration of *A. baumannii* ATCC17978ΔabaI resulted in 70% mortality after 24 h (Figure 4; *p* < 0.05, Mantel-Cox analysis).

**DISCUSSION**

In this study, we analyzed the expression of Quorum network (QS/QO) genes that differed between genomes of clinical isolates of *A. baumannii*, abaR and abaI (QS system) and aidA (QQ mechanism) in relation to clinical features of pneumonia and bacteremia. Although other QQ enzymes have been described in *A. baumannii* ATCC 17978 (Mayer et al., 2018), these were not analyzed in the present study due to the lack of any differences between *A. baumannii* genomes.

In clinical strains of *A. baumannii* isolated from patients with bacteraemic pneumonia (Pn-B), the abaR gene was overexpressed (*p* < 0.05). The AbaR protein was the receptor activator of the Quorum Sensing system (QS), and the aidA gene was not expressed. Moreover, we observed regulation of aidA gene expression in clinical strains of pneumonia-causing *A. baumannii* (non-bacteraemic pneumonia, Pn-NB) by the 3-Oxo-C12-HSL molecule (which is an AidA enzyme substrate in QQ activity) and H₂O₂ (an activator of the QS system). However, there was no difference in the expression of Quorum network genes between colonized and pneumonia patients, as previously described (Stones and Krachler, 2016).

On the other hand, clinical analysis of the risk factors associated with pneumonia caused by *A. baumannii* revealed diabetes mellitus as only statistically significant risk factor (Kim et al., 2014). In relation to bacteremia in *A. baumannii* pneumonia (*P* < 0.05), underexpression of the aidA gene was also the only statistically significant variable (*P* < 0.05).

In several pathogens, such as *Yersinia pseudotuberculosis*, *Proteus mirabilis*, and *Vibrio cholerae*, the QS system is the main regulatory mechanism of bacterial competence via T6SS, which is involved in the invasiveness and motility that favor the development of bacteremia (Zhang et al., 2011; Debnath et al., 2018; Jaskólska et al., 2018; Trastoy et al., 2018). Moreover, in 86% of ICU patients, gastrointestinal tract colonization by a clinical strain of *A. baumannii* led to development of bacteremia caused by genetically similar strains (Thom et al., 2010). This implies that clinical isolates of *A. baumannii* most capable of...
Figure 2 | Relative expression of the abaR and aidA genes under 3-oxo-C12-HSL (A) and H₂O₂ (B) in isolates of A. baumannii from patients with non-bacteraemic pneumonia (Pn-NB). *p-value < 0.05.

Figure 3 | Relative expression of the abaR and aidA genes under 3-oxo-C12-HSL (A) and H₂O₂ (B) in isolates of A. baumannii from patients with bacteraemic pneumonia (Pn-B). *p-value < 0.05.
surviving under stress conditions (such as the presence of bile salts in the gastrointestinal tract or H₂O₂ in the respiratory tract) (Zheng et al., 2018) may have a higher invasive capacity due to virulence factors, such as the type VI secretion system (T6SS), previously activated under stressful conditions. Motility is also a crucial virulence factor, allowing penetration of the bacteria into the host’s body and subsequent colonization (Gellatly and Hancock, 2013). Previous studies have demonstrated the existence of a relationship between motility and the origin of the isolates. Indeed, blood isolates of A. baumannii have been found to be more mobile than sputum isolates (Jaskólska et al., 2018).

In conclusion, our findings suggest that the QS (abaR and abaI genes)/QQ (aidA gene) network plays a role in the development of bacteremia in patients with pneumonia caused by A. baumannii. This is the first study reporting a relationship between reduced expression of this bacterial QQ enzyme gene (AidA protein) and bacteremia. Further studies are needed to validate these findings and to understand the underlying mechanisms.
of this relationship in the same and other bacterial QQ enzymes would be of great interest.

AUTHOR CONTRIBUTIONS

LF-G, AA, LB, IB, ML and RA-M developed the experiments. FF-C, LM-M, JV, JR-B, JG-M, JMC, AP, JP, GB, and YS wrote the manuscript and provided the strains. MT led the experiments and manuscript redaction.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.03105/full#supplementary-material

REFERENCES

Bhargava, N., Sharma, P., and Capalash, N. (2012). N-acyl homoserine lactone mediated interspecies interactions between A. baumannii and P. aeruginosa. Biofouling. 28, 813–822. doi: 10.1080/08927014.2012.714372

Bone, R. C., Balk, R. A., Cerra, F. B., Dellinger, R. P., Fein, A. M., Knaus, W. A., et al. (1992). Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest H101, 1644–1655. doi: 10.1378/chest.101.6.1644

Castañeda-Tamez, P., Ramírez-Peris, J., Pérez-Velázquez, J., Kuttler, C., Jalalimanesh, A., Saucedo-Mora, M. Á., et al. (2018). Pyocyanin restricts social cheating in Pseudomonas aeruginosa. Front. Microbiol. 9:1348. doi: 10.3389/fmicb.2018.01348

Charlson, M. E., Pompei, P., Ales, K. L., and MacKenzie, C. R. (1987). A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J. Chronic Dis. 40, 373–383. doi: 10.1016/0021-9681(87)90171-8

Cisneros, J. M., and Rodriguez-Baño, J. (2002). Nosocomial bacteremia due to Acinetobacter baumannii: epidemiology, clinical features and treatment. Clin. Microbiol. Infect. 8, 687–693. doi: 10.1046/j.1469-0691.2002.00487.x

Debnath, I., Stringer, A. M., Smith, S. N., Bae, E., Mobley, H. L. T., Wade, J. T., et al. (2018). MrpJ directly regulates proteus mirabilis virulence factors, including fimbriae and type VI secretion, during urinary tract infection. Infect Immun. 86, e00388–18. doi: 10.1128/IAI.00388-18

del Mar Tomas, M., Cartelle, M., Pertega, S., Beceiro, A., Linares, P., Canle, D., et al. (2005). Hospital outbreak caused by a carbapenem-resistant strain of Acinetobacter baumannii: patient prognosis and risk-factors for colonisation and infection. Clin. Microbiol. Infect. 11, 540–546. doi: 10.1111/j.1469-0691.2005.01184.x

El Kettani, A., Maaloum, F., Diawara, I., Katfy, K., Harrar, N., Zerouali, K., et al. (2017). Prevalence of Acinetobacter baumannii bacteremia in intensive care units of Ibn Rochd University Hospital, Casablanca. Iran J Microbiol. 9, 318–323.

Fernández-Cuenca, F., Tomás, M., Caballero-Moyano, F. J., Bou, G., Martínez-Martínez, L., Vila, J., et al. (2015). Reduced susceptibility to biocides in Acinetobacter baumannii: association with resistance to antimicrobials, epidemiological behaviour, biological cost and effect on the expression of genes
Yang, H., Chen, G., Hu, L., Liu, Y., Cheng, J., Li, H., et al. (2015). In vivo activity of daptomycin/colistin combination therapy in a Galleria mellonella model of Acinetobacter baumannii infection. Int. J. Antimicrob. Agents 45, 188–191. doi: 10.1016/j.ijantimicag.2014.10.012

Zhang, W., Xu, S., Li, J., Shen, X., Wang, Y., and Yuan, Z. (2011). Modulation of a thermoregulated type VI secretion system by AHL-dependent quorum sensing in Yersinia pseudotuberculosis. Arch. Microbiol. 193, 351–363. doi: 10.1007/s00203-011-0680-2

Zheng, Y., Li, Y., Long, H., Zhao, X., Jia, K., Li, J., et al. (2018). bifA regulates biofilm development of Pseudomonas putida MnB1 as a primary response to H2O2 and Mn2+. Front. Microbiol. 9:1490. doi: 10.3389/fmicb.2018.01490

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