**Supplementary Material**

**Relationship between antibiotic resistance, efflux pumps, and biofilm formation in extended-spectrum β-lactamase producing *Klebsiella pneumoniae***

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**Supplementary Tables**

**Table S1 Zone diameter distribution of isolates against different antibiotics**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Zone Diameter (mm) | | | | | | | | | | | | | | | | | | |  |
| Antibioticsa | 0-2c | 2-4 | 4-6 | 6-8 | 8-10 | 10-12 | 12-14 | 14-16 | 16-18 | 18-20 | 20-22 | 22-24 | 24-26 | 26-28 | 28-30 | 30-32 | 32-34 | 34-36 | 36-38 | 38-40 |
| AM | 100b | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CAZ | 44 | 0 | 0 | 0 | 1 | 5 | 7 | 9 | 6 | 7 | 7 | 2 | 5 | 3 | 3 | 0 | 1 | 0 | 0 | 0 |
| CTX | 81 | 0 | 0 | 1 | 4 | 8 | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| CRO | 81 | 0 | 0 | 2 | 10 | 3 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ATM | 55 | 0 | 0 | 0 | 0 | 3 | 8 | 7 | 6 | 4 | 5 | 5 | 2 | 2 | 1 | 2 | 0 | 0 | 0 | 0 |
| CIP | 47 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 5 | 9 | 6 | 3 | 4 | 3 | 4 | 6 | 5 | 0 | 2 | 1 |
| ETP | 15 | 0 | 0 | 0 | 0 | 2 | 7 | 4 | 3 | 1 | 7 | 3 | 14 | 9 | 10 | 15 | 8 | 2 | 0 | 0 |
| TOB | 22 | 0 | 1 | 5 | 15 | 25 | 6 | 6 | 5 | 8 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| TET | 57 | 0 | 0 | 0 | 1 | 6 | 7 | 6 | 3 | 10 | 7 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

a AM, ampicillin; CAZ, ceftazidime; CTX, cefotaxime; CRO, ceftriaxone; ATM, aztreonam; CIP, ciprofloxacin; ETP, ertapenem; TOB, tobramycin; TET, tetracycline.

bNumber of isolates.

cZone diameter range.

**Table S2 Antibiotic susceptibilities of isolates and fold changes of the efflux pumps**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | Antibiotic susceptibilitya | | | | | | | | | Fold change in gene expressione | | | | | |
| Strain | | CAZ | CTX | CRO | ATM | CIP | ETP | NN | AM | TE | *acrA* | *ketM* | *kdeA* | *kpnEF* | *kexD* | |
| **1** | | Rb | R | R | R | R | R | R | R | R | 0,15 | 0,60 | 2,11↑ | 1,74↑ | 47,84↑ | |
| **2** | | R | R | R | R | Ic | R | Sd | R | R | 0,11 | 0,71 | 2,20↑ | 2,17↑ | NDf | |
| **3** | | R | R | R | R | R | I | R | R | R | 0,03 | 0,13 | 0,48 | 0,44 | 2,01 | |
| **4** | | R | R | R | R | I | R | I | R | R | 0,06 | 0,31 | 1,14↑ | 0,88 | ND | |
| **5** | | I | R | R | R | S | S | R | R | R | 0,00 | 0,12 | 0,43 | 0,20 | 0,00 | |
| **6** | | R | R | R | R | I | R | I | R | R | 0,04 | 0,30 | 0,90 | 0,66 | ND | |
| **7** | | S | R | R | S | S | I | R | R | S | 0,04 | 0,09 | 0,82 | 0,50 | 3,23↑ | |
| **8** | | R | R | R | R | R | I | R | R | R | 0,05 | 0,32 | 1,58↑ | 0,68 | 4,92↑ | |
| **9** | | R | R | R | R | I | S | R | R | I | 0,03 | 0,18 | 0,60 | 0,30 | ND | |
| **10** | | R | R | R | R | S | S | R | R | R | 0,02 | 0,08 | 0,33 | 0,13 | 0,61 | |
| **11** | | R | R | R | R | R | S | R | R | R | 0,41 | 2,39↑ | 6,50↑ | 4,35↑ | 0,02 | |
| **12** | | R | R | R | R | R | I | R | R | R | 0,05 | 0,15 | 0,58 | 0,24 | 1,72↑ | |
| **13** | | R | R | R | R | R | S | R | R | R | 0,11 | 0,32 | 2,11↑ | 0,56 | 5,35↑ | |
| **14** | | R | R | R | R | R | S | R | R | I | 0,70 | 3,97↑ | 17,39↑ | 4,82↑ | 39,67↑ | |
| **15** | | R | R | R | R | R | I | I | R | R | 0,03 | 0,13 | 0,54 | 0,29 | 2,16↑ | |
| **16** | | R | R | R | S | S | S | S | R | R | 1,51↑ | 16,80↑ | 55,72↑ | 19,03↑ | 0,01 | |
| **17** | | R | R | R | R | S | S | S | R | I | 2,19↑ | 30,70↑ | 25,11↑ | 35,02↑ | 4,26↑ | |
| **18** | | S | R | R | S | S | S | I | R | R | 0,34 | 2,79↑ | 13,27↑ | 6,77↑ | ND | |
| **19** | | R | R | R | R | R | S | R | R | R | 0,22 | 3,01↑ | 6,54↑ | 1,99↑ | 19,43↑ | |
| **20** | | R | R | R | R | S | R | R | R | I | 0,26 | 3,36↑ | 9,51↑ | 3,36↑ | 22,32↑ | |
| **21** | | R | R | R | R | R | S | R | R | R | 0,26 | 2,39↑ | 6,54↑ | 3,36↑ | 15,89↑ | |
| **22** | | I | R | R | I | S | S | S | R | R | 0,10 | 1,24↑ | 4,66↑ | 1,73↑ | 9,99↑ | |
| **23** | | R | R | R | R | R | R | R | R | R | 0,73 | 14,52↑ | 22,32↑ | 15,35↑ | 120,26↑ | |
| **24** | | I | R | R | R | S | S | R | R | R | 0,14 | 2,35↑ | 6,11↑ | 3,12↑ | ND | |
| **25** | | I | R | R | R | R | S | R | R | R | 0,29 | 2,46↑ | 11,96↑ | 3,78↑ | ND | |
| **26** | | S | R | R | I | S | S | S | R | S | 0,01 | 16,22↑ | 35,75↑ | 57,68↑ | 0,70 | |
| **27** | | S | R | R | S | S | S | S | R | S | 2,23↑ | 6,32↑ | 20,11↑ | 9,25↑ | 1,35↑ | |
| **28** | | R | R | R | R | I | S | R | R | R | 0,70 | 8,34↑ | 9,99↑ | 5,74↑ | 0,02 | |
| **29** | | R | R | R | R | I | S | R | R | R | 1,15↑ | 10,78↑ | 18,25↑ | 7,57↑ | ND | |
| **30** | | S | R | R | S | R | S | S | R | S | 0,00 | 0,04 | 0,00 | 0,00 | 0,06 | |
| **31** | | S | R | R | S | S | S | S | R | S | 1,04↑ | 56,49↑ | 104,69↑ | 83,29↑ | ND | |
| **32** | | R | R | R | R | R | S | R | R | S | 0,61 | 8,75↑ | 19,43↑ | 11,88↑ | 33,82↑ | |
| **33** | | R | R | R | R | S | S | R | R | R | 2,19↑ | 10,56↑ | 43,41↑ | 11,79↑ | 87,43↑ | |
| **34** | | R | R | R | R | R | R | R | R | S | 1,35↑ | 18,25↑ | 31,34↑ | 36,25↑ | 97,01↑ | |
| **35** | | S | R | R | S | S | S | R | R | S | 2,25↑ | 21,71↑ | 44,32↑ | 13,64↑ | 85,04↑ | |
| **36** | | R | R | R | R | R | R | R | R | S | 1,25↑ | 20,82↑ | 30,27↑ | 29,45↑ | 183,55↑ | |
| **37** | | R | R | R | R | R | R | R | R | R | 1,15↑ | 19,84↑ | 34,78↑ | 13,93↑ | ND | |
| **38** | | R | R | R | R | S | S | S | R | S | 1,87↑ | 14,93↑ | 40,50↑ | 26,54↑ | 1,21↑ | |
| **39** | | R | R | R | R | R | R | R | R | R | 1,22↑ | 13,36↑ | 32,67↑ | 11,31↑ | ND | |
| **40** | | R | R | R | R | R | S | R | R | I | 0,85 | 14,32↑ | 25,99↑ | 20,68↑ | 135,30↑ | |
| **41** | | R | R | R | R | R | R | R | R | R | 0,61 | 6,32↑ | 14,72↑ | 6,02↑ | ND | |
| **42** | | R | R | R | R | R | S | R | R | R | 1,16↑ | 13,83↑ | 34,78↑ | 14,32↑ | ND | |
| **43** | | R | R | R | R | S | I | S | R | S | 0,31 | 2,25↑ | 5,70↑ | 3,23↑ | 12,91↑ | |
| **44** | | R | R | R | R | R | R | R | R | S | 1,15↑ | 13,00↑ | 28,84↑ | 13,27↑ | ND | |
| **45** | | R | R | R | R | R | R | R | R | R | 0,11 | 1,13↑ | 3,63↑ | 0,47 | ND | |
| **46** | | R | R | R | R | R | S | R | R | I | 0,33 | 3,53↑ | 6,87↑ | 2,10↑ | 20,25↑ | |
| **47** | | R | R | R | R | R | S | S | R | I | 0,50 | 3,01↑ | 7,46↑ | 2,53↑ | 19,16↑ | |
| **48** | | R | R | R | R | R | R | R | R | R | 0,21 | 1,78↑ | 7,26↑ | 1,49↑ | ND | |
| **49** | | R | R | R | R | R | R | R | R | R | 0,18 | 3,25↑ | 10,06↑ | 1,80↑ | ND | |
| **50** | | R | R | R | R | R | R | R | R | R | 0,02 | 0,22 | 0,46 | 0,28 | 0,01 | |
| **51** | | R | R | R | R | R | S | R | R | R | 0,04 | 0,26 | 0,73 | 0,47 | ND | |
| **52** | | R | R | R | R | R | R | R | R | R | 0,01 | 0,05 | 0,23 | 0,07 | ND | |
| **53** | | R | R | R | R | S | S | R | R | R | 0,14 | 0,74 | 1,91↑ | 0,61 | 4,56↑ | |
| **54** | | R | R | R | R | R | R | R | R | R | 0,01 | 0,05 | 0,26 | 0,07 | ND | |
| **55** | | R | R | R | R | R | R | R | R | R | 0,12 | 1,01↑ | 3,03↑ | 2,04↑ | 11,63↑ | |
| **56** | | S | R | R | S | S | S | S | R | R | 0,00 | 0,04 | 0,29 | 0,14 | ND | |
| **57** | | R | R | R | R | R | S | S | R | S | 0,07 | 0,37 | 1,92↑ | 0,81 | 1,48↑ | |
| **58** | | I | R | R | R | R | S | R | R | R | 0,44 | 2,36↑ | 11,16↑ | 4,56↑ | 0,31 | |
| **59** | | S | R | R | S | S | S | R | R | R | 0,38 | 0,75 | 7,26↑ | 1,56↑ | 0,05 | |
| **60** | | S | R | R | S | I | S | R | R | R | 0,24 | 0,90 | 5,70↑ | 1,62↑ | 9,92↑ | |
| **61** | | S | R | R | S | I | S | R | R | R | 0,07 | 0,25 | 1,62↑ | 0,31 | 2,01↑ | |
| **62** | | R | R | R | R | R | R | R | R | R | 0,29 | 0,95 | 4,17↑ | 1,42↑ | ND | |
| **63** | | R | R | R | R | R | R | R | R | R | 0,15 | 0,32 | 2,81↑ | 0,36 | ND | |
| **64** | | R | R | R | R | R | R | R | R | R | 0,16 | 0,44 | 3,20↑ | 0,88 | ND | |
| **65** | | R | R | R | R | R | S | R | R | S | 0,17 | 0,29 | 2,31↑ | 0,85 | 0,01 | |
| **66** | | R | R | R | R | R | R | R | R | I | 0,56 | 2,04↑ | 8,82↑ | 5,46↑ | 23,43↑ | |
| **67** | | S | R | R | S | S | S | S | R | S | 0,26 | 0,82 | 2,73↑ | 0,72 | 3,25↑ | |
| **68** | | R | R | R | R | S | S | S | R | S | 3,25↑ | 8,40↑ | 31,56↑ | 19,97↑ | 21,71↑ | |
| **69** | | S | R | R | I | I | S | R | R | R | 2,50↑ | 8,06↑ | 23,10↑ | 15,24↑ | 313,00↑ | |
| **70** | | S | R | R | S | S | S | S | R | S | 0,17 | 0,43 | 2,01↑ | 0,64 | 3,34↑ | |
| **71** | | I | R | R | I | I | S | S | R | R | 0,82 | 0,78 | 6,82↑ | 1,22↑ | 43,71↑ | |
| **72** | | S | R | R | S | S | S | S | R | R | 0,02 | 0,65 | 3,94↑ | 0,59 | 5,58↑ | |
| **73** | | S | R | R | S | S | S | R | R | R | 0,08 | 0,14 | 1,38↑ | 0,35 | 0,00 | |
| **74** | | R | R | R | R | R | R | R | R | R | 0,07 | 0,34 | 1,87↑ | 0,31 | 0,00 | |
| **75** | | I | R | R | I | I | S | R | R | R | 0,02 | 0,07 | 0,31 | 0,07 | 0,77 | |
| **76** | | R | R | R | R | R | S | R | R | I | 0,07 | 0,95 | 4,32↑ | 1,10↑ | 60,97↑ | |
| **77** | | R | R | R | R | R | R | R | R | I | 0,44 | 5,21↑ | 18,00↑ | 15,78↑ | 224,41↑ | |
| **78** | | R | R | R | R | R | S | R | R | R | 2,07↑ | 25,81↑ | 57,28↑ | 28,44↑ | ND | |
| **79** | | I | R | R | R | R | S | R | R | R | 0,01 | 0,09 | 0,33 | 0,12 | ND | |
| **80** | | I | R | R | R | R | R | R | R | R | 0,07 | 0,39 | 2,10↑ | 0,40 | 0,00 | |
| **81** | | I | R | R | R | S | S | R | R | S | 0,00 | 1,05↑ | 4,82↑ | 1,52↑ | 10,27↑ | |
| **82** | | R | R | R | R | S | R | R | R | R | 1,46↑ | 5,46↑ | 35,26↑ | 13,27↑ | 144,01↑ | |
| **83** | | R | R | R | R | I | S | R | R | R | 0,01 | 58,49↑ | 404,50↑ | 179,77↑ | ND | |
| **84** | | R | R | R | R | I | S | R | R | S | 0,27 | 1,85↑ | 9,25↑ | 2,00↑ | 13,45↑ | |
| **85** | | R | R | R | R | I | S | R | R | R | 0,22 | 0,67 | 5,28↑ | 1,53↑ | 7,52↑ | |
| **86** | | R | R | R | R | R | R | R | R | R | 0,20 | 1,28↑ | 7,57↑ | 2,06 | 0,00 | |
| **87** | | R | R | R | R | I | S | R | R | S | 0,04 | 0,34 | 1,16↑ | 0,51 | ND | |
| **88** | | S | R | R | S | S | S | S | R | S | 0,04 | 0,21 | 1,01↑ | 0,45 | 2,68↑ | |
| **89** | | R | R | R | R | R | R | R | R | R | 0,12 | 0,90 | 3,92↑ | 0,89 | ND | |
| **90** | | R | R | R | R | I | S | R | R | S | 0,03 | 0,10 | 0,50 | 0,13 | ND | |
| **91** | | R | R | R | R | R | S | R | R | R | 0,02 | 0,18 | 0,56 | 0,15 | 1,08↑ | |
| **92** | | R | R | R | R | R | R | S | R | R | 0,04 | 0,23 | 1,06↑ | 0,18 | ND | |
| **93** | | R | R | R | R | R | R | R | R | R | 1,64↑ | 4,92↑ | 25,28↑ | 11,08↑ | ND | |
| **94** | | R | R | R | R | R | R | R | R | R | 0,28 | 1,87↑ | 8,51↑ | 1,62↑ | 0,07 | |
| **95** | | R | R | R | R | I | S | R | R | R | 0,42 | 1,27↑ | 5,46↑ | 0,98 | 18,77↑ | |
| **96** | | R | R | R | R | R | S | R | R | S | 0,54 | 1,21↑ | 7,16↑ | 1,08↑ | 6,68↑ | |
| **97** | | R | R | R | R | S | S | I | R | R | 1,97↑ | 4,82↑ | 18,64↑ | 6,41↑ | 48,17↑ | |
| **98** | | R | R | R | R | R | S | R | R | R | 1,52↑ | 4,47↑ | 32,00↑ | 7,78↑ | 37,53↑ | |
| **99** | | R | R | R | R | R | S | R | R | S | 1,87↑ | 4,69↑ | 28,25↑ | 12,21↑ | 76,64↑ | |
| **100** | | S | R | R | R | S | S | S | R | S | 0,08 | 0,70 | 4,20↑ | 0,70 | 0,63 | |
| a AM, Ampicillin; CAZ, Ceftazidime; CTX, Cefotaxime; CRO, Ceftriaxone; ATM, aztreonam; CIP, ciprofloxacin; ETP, ertapenem; TOB, tobramycin; TET, tetracycline.  b Resistant strains  c Intermediate strains  d Susceptible strains  e Fold change was calculated by 2-∆∆Ct method  f ND,data not determined  ↑ Up-regulated gene expression, each gene was normalized to relative expression of *Klebsiella pneumoniae* ATCC 700721, which was assigned a value of 1.0 | | | | | | | | | | | | | | |

**Table S3 Biofilm formation of *Klebsiella pneumoniae* isolates**

|  |  |  |
| --- | --- | --- |
| **Weakb** | **Moderatec** | **Strongd** |
| 3 (OD570a = 0,093) | 11 (OD570a = 0,252) | 1 (OD570a = 0,330) |
| 28 (OD570a = 0,142) | 12 (OD570a = 0,226) | 2 (OD570a = 1,005) |
| 29 (OD570a = 0,126) | 15 (OD570a = 0,228) | 4 (OD570a = 0,840) |
| 39 (OD570a = 0,142) | 24 (OD570a = 0,312) | 5 (OD570a = 0,512) |
| 41 (OD570a = 0,127) | 27 (OD570a = 0,230) | 6 (OD570a = 1,036) |
| 48 (OD570a = 0,096) | 31 (OD570a = 0,217) | 7 (OD570a = 0,494) |
| 51 (OD570a = 0,086) | 32 (OD570a = 0,246) | 8 (OD570a = 0,600) |
| 57 (OD570a = 0,087) | 37 (OD570a = 0,206) | 9 (OD570a = 1,511) |
| 58 (OD570a = 0,086) | 49 (OD570a = 0,240) | 10 (OD570a = 0,365) |
| 63 (OD570a = 0,110) | 62 (OD570a = 0,252) | 13 (OD570a = 0,416) |
| 69 (OD570a = 0,114) | 64 (OD570a = 0,253) | 14 (OD570a = 0,330) |
| 80 (OD570a = 0,161) | 66 (OD570a = 0,249) | 16 (OD570a = 0,505) |
| 85 (OD570a = 0,152) | 74 (OD570a = 0,272) | 17 (OD570a = 0,385) |
| 94 (OD570a = 0,097) | 79 (OD570a = 0,191) | 18 (OD570a = 0,369) |
| 96 (OD570a = 0,152) | 83 (OD570a = 0,232) | 19 (OD570a = 0,395) |
| 99 (OD570a = 0,090) | 92 (OD570a = 0,231) | 20 (OD570a = 0,375) |
|  |  | 21 (OD570a = 0,469) |
|  |  | 22 (OD570a = 0,682) |
|  |  | 23 (OD570a = 0,782) |
|  |  | 25 (OD570a = 0,785) |
|  |  | 26 (OD570a = 0,633) |
|  |  | 30 (OD570a = 0,666) |
|  |  | 33 (OD570a = 0,685) |
|  |  | 34 (OD570a = 0,353) |
|  |  | 35 (OD570a = 0,335) |
|  |  | 36 (OD570a = 0,442) |
|  |  | 38 (OD570a = 1,256) |
|  |  | 40 (OD570a = 0,331) |
|  |  | 42 (OD570a = 0,477) |
|  |  | 43 (OD570a = 0,543) |
|  |  | 44 (OD570a = 1,318) |
|  |  | 45 (OD570a = 0,444) |
|  |  | 46 (OD570a = 0,920) |
|  |  | 50 (OD570a = 0,963) |
|  |  | 52 (OD570a = 0,332) |
|  |  | 53 (OD570a = 1,322) |
|  |  | 54 (OD570a = 0,331) |
|  |  | 55 (OD570a = 0,919) |
|  |  | 56 (OD570a = 0,336) |
|  |  | 59 (OD570a = 0,402) |
|  |  | 60 (OD570a = 0,341) |
|  |  | 61 (OD570a = 0,650) |
|  |  | 65 (OD570a = 0,526) |
|  |  | 67 (OD570a = 0,346) |
|  |  | 68 (OD570a = 0,705) |
|  |  | 70 (OD570a = 0,343) |
|  |  | 71 (OD570a = 0,917) |
|  |  | 72 (OD570a = 0,571) |
|  |  | 73 (OD570a = 0,390) |
|  |  | 75 (OD570a = 0,691) |
|  |  | 76 (OD570a = 1,327) |
|  |  | 77 (OD570a = 0,450) |
|  |  | 78 (OD570a = 0,364) |
|  |  | 81 (OD570a = 1,512) |
|  |  | 82 (OD570a = 2,312) |
|  |  | 84 (OD570a = 0,610) |
|  |  | 86 (OD570a = 0,744) |
|  |  | 87 (OD570a = 0,453) |
|  |  | 88 (OD570a = 0,499) |
|  |  | 89 (OD570a = 0,363) |
|  |  | 90 (OD570a = 0,457) |
|  |  | 91 (OD570a = 0,425) |
|  |  | 93 (OD570a = 0,579) |
|  |  | 95 (OD570a = 0,486) |
|  |  | 97 (OD570a = 0,327) |
|  |  | 98 (OD570a = 0,456) |
|  |  | 100 (OD570a = 0,496) |

a Optical density at 570 nm.

b 0.081 (ODcontrol) < ODsample < 0.162 (2xODcontrol)

c 0.162(2xODcontrol) < ODsample < 0.324 (4xODcontrol)

d 0.324 (4xODcontrol) < ODsample

e No biofilm formation (ODsample < 0.081 (ODcontrol)