

# Burden of disease associated with antimicrobial resistance

Kraker, Marlieke Elizabeth Adriana de

# 4

# Clinical effectiveness of antibiotic therapy in *Staphylococcus aureus* and *Escherichia coli* bloodstream infections: An observational cohort study in European hospitals

M.E.A. de Kraker[1,2], M. Wolkewitz[3], P.G. Davey[4], W. Koller[5], J. Berger[5], J. Nagler[6], C. Icket[6], S. Kalenic[7], J. Horvatic[7], H. Seifert[8], A. Kaasch[8], O. Paniara[9], A. Argyropoulou[9], M. Bompola[9], E. Smyth[10], M. Skally[10], A. Raglio[11], U. Dumpis[12], A. Melbarde Kelmere[12], M. Borg[13], D. Xuereb[13], M.C. Ghita[14], M. Noble[15], J. Kolman[16], S. Grabljevec[17], D. Turner[18], L. Lansbury[18], H. Grundmann[1,2]

[1]Centre for Infectious Disease Control, RIVM, Bilthoven, The Netherlands; [2]Department of Medical Microbiology, UMCG, Groningen, The Netherlands; [3]Institute of Medical Biometry and Medical Informatics, University Medical Centre Freiburg, Freiburg, Germany; [4]Division of Community Health Sciences, Health Informatics Centre, Dundee, UK; [5]Clinical Institute for Hospital Hygiene, Medical University of Vienna and Vienna General Hospital, Vienna, Austria; [6]Algemeen Ziekenhuis Middelheim ZNA, Antwerp, Belgium; [7]Department of Clinical and Molecular Microbiology, University Hospital Centre and University of Zagreb, Zagreb, Croatia; [8]Institute for Medical Microbiology, Immunology and Hygiene, University of Cologne, Cologne, Germany; [9]Evangelismos General Hospital, Athens, Greece; [10]Microbiology Department, Beaumont Hospital, Dublin, Ireland; [11]USC Microbiologia e Virologia, AO Ospedali Riuniti, Bergamo, Italy; [12]Department of Infection Control, Stradins University Hospital, Riga, Latvia; [13]Infection Control Unit, Mater Dei Hospital, Msida, Malta; [14]Clinical Institute Fundeni, Bucharest, Romania; [15]Surgical Pre-Assessment Clinic, Day Surgery Unit, Ninewells Hospital, Dundee, UK; [16]National Institute of Public Health, Ljubljana, Slovenia; [17]UMC Ljubljana, Ljubljana, Slovenia; [18]Queen's Medical Centre Campus, Nottingham University Hospitals NHS Trust, Nottingham, UK

Submitted

# Abstract

## Background

Clinical effectiveness of antibiotic therapy is dependent on the timely administration of *in vitro* active antibiotic therapy (appropriate therapy). In this observational study we ascertained the time to appropriate therapy and its effect on 30-day survival in patients with BSIs caused by *S. aureus* or *E. coli*.

## Methods

For this prospective cohort study patients were enrolled from thirteen tertiary care hospitals in as many European countries between June 2007 and June 2008. The impact of appropriate therapy was analysed by survival analysis with appropriate therapy included as time-dependent variable.

## Results

In total, 763 and 1,028 patients with *S. aureus* and *E. coli* BSIs were included. In the *S. aureus* cohort, 91% patients received appropriate therapy, whereby patients with MSSA received appropriate therapy significantly earlier (after a median of 4 hours) than patients with MRSA (after a median of 48 hours). In the *E. coli* cohort, 93% of the patients received appropriate therapy. Again, patients infected with SEC received appropriate therapy significantly earlier (median 4 hours) than patients with resistant isolates (median 40 hours). Compared to *in vitro* inactive antibiotic therapy, appropriate therapy significantly reduced mortality in the MSSA (HR, 0.47) and SEC (HR, 0.38) cohort, but not in patients with resistant infections.

## Conclusions

In European hospitals, time to appropriate therapy in patients with susceptible BSIs is short, improving survival. When infections are complicated by resistance, administration of appropriate therapy is often delayed and not improving survival.

# Introduction

Antimicrobial chemotherapy that is active against pathogens causing infections is essential for the success of modern medical interventions. The potential beneficial effect is, however, dependent on timely administration of the correct antibiotic compound. Lack or misjudgement of clinical symptoms of infection can lead to unwarranted delays in antibiotic treatment. At the same time, the range of possible etiological agents as well as antimicrobial resistance can even further protract the selection of a compound with the correct spectrum of activity.

Time until administration of appropriate therapy could influence infection related mortality. This may in part explain why patients suffering from infections caused by antibiotic resistant bacteria experience higher mortality than patients infected with susceptible bacteria.[1-4] We carried out the current investigation, in order to determine whether the prevalent antibiotic treatment practice in European tertiary care centres can explain the observed differences in survival between these groups of patients.

When estimating the clinical impact of appropriate therapy in observational studies, treatment independent survival can lead to bias. Infected patients surviving for a longer period have a higher chance of receiving appropriate therapy than patients dying shortly after diagnosis. This is due to the fact that informed adjustment of antibiotic therapy can only take place after results from the microbiological laboratory have become available. This could lead to the foregone conclusion that patients receiving appropriate therapy have a better chance of survival even if therapy did not have an effect. Time-to-event analysis can eliminate bias due to this potential spurious association between appropriate therapy and survival, known as immortal time bias.[5]

In this article we describe a prospective cohort study, in which we observed the time to administration of appropriate therapy and its effect on 30 day survival for patients with a BSI caused by susceptible versus resistant *S. aureus* or *E. coli*, using time-dependent analysis.

## Material and methods

A prospective cohort study was carried out in thirteen tertiary care hospitals in as many different European countries between June 2007 and June 2008. All hospitals were participating in the EARSS and were selected on the basis of reliable diagnostic quality of their microbiological laboratories and representative levels of resistance for their countries.[6] Details can be found elsewhere.[3,4]

The cohorts consisted of patients with microbiologically confirmed *S. aureus* or *E. coli* BSI. Patients were identified by daily liaison with the microbiological laboratory. Criteria for inclusion consisted of: i) ≥ 18 years, ii) mono-microbial BSI, iii) antibiotic therapy started after the index blood culture, iv) survival for at least one day after the index blood culture.

Methods of data collection were described in detail elsewhere.[3,4] The following individual information was collected between admission and the index blood culture: Date of admission, patient referral history (community, long-term care facility, nursing home or other hospital admission), frequent hospital exposure (defined as two or more hospital admissions in the previous 12 months), type of admission (emergency or elective), surgery, presence of co-morbidities from the CCI[7], and antibiotic therapy. Timing of the blood culture was recorded in hours and minutes, at the same day presence of indwelling devices (tracheal tube, central venous catheter, arterial vascular access, peripheral vascular access, urinary catheter, tracheostomy, nasogastric tube, wound drainage tube) and treatment in ICU were recorded. The anatomical origin of the BSI (in the case of secondary BSI) and the susceptibility profile of the causative pathogen were extracted from the laboratory information system. All antibiotic regimens, administered after the positive blood culture, were registered, including time (hours and minutes) and date of administration.

Empirical therapy was defined as all therapy received within the first two days after the index blood culture. Appropriate therapy was defined as administration of an antibiotic compound for which the identified bacterial isolate showed clinical susceptibility as tested *in vitro* by the diagnostic laboratory. For MRSA and G3CREC class resistance against all beta-lactam agents, except carbapenems in the case of G3CREC, was assumed. The main outcome was mortality 30 days after the index blood culture with follow-up beyond hospital discharge.

This study complied with the Dutch patient confidentiality regulations and ethical standards and was approved by local institutional ethical committees when required.

### Statistical analysis

Statistical analyses were performed using SAS 9.1 and R 2.8.1. All analyses were performed separately for the *S. aureus* and *E. coli* cohort. For comparison of patient characteristics Chi-square or Mann-Whitney U tests were used for dichotomous and continuous variables.

Kaplan-Meier curves were plotted to compare the time (in hours) to appropriate therapy between patients with MSSA versus MRSA, or those with a SEC versus G3CREC. The difference was tested by a signed log-rank test.

The association between time, appropriate therapy and clinical outcome was illustrated in a bar chart, whereby mortality rates (per day) were calculated for each day since the index blood culture. Mortality rates were given separately for patients with and without appropriate therapy, whereby patients shifted from the "no appropriate therapy" group to the "appropriate therapy" group on the day appropriate therapy was initiated. The overall clinical effectiveness of appropriate therapy was analysed by survival analysis. To adjust for the time between blood culture and appropriate therapy, appropriate therapy was included as a time-dependent variable, thereby eliminating possible immortal time bias.[5] Multivariate models were used to adjust for differences between patients with and without appropriate therapy. Variables were included if they changed the effect estimate in bivariate regression by more than 5%. The likelihood ratio test was used to select the model with the best fit by backward selection, whereby $p<0.05$ was considered significant. Proportionality for all included variables was checked with the Wald test for time interaction. In the final model, interaction was tested for all clinically relevant combinations. To correct for cluster effects within hospitals we used stratified analysis when the estimate for appropriate therapy changed more than 5% after stratification. For all estimates $CI_{95}$ were reported.

## Results

Between June 2007 and June 2008, relevant BSIs were recorded in 13 hospitals during 4,791,550 patient-days.[3,4] Altogether, 1,000 and 1,328 patients were identified with *S. aureus* and *E. coli* BSIs, respectively. Of these 763 (76%) and 1,028 (77%), fulfilled the inclusion criteria.

In the *S. aureus* cohort, the median age was 68 (IQR, 55-77). Two hundred twenty-eight of 763 (30%) patients had MRSA. The majority was male (466, 61%) and admitted through the emergency department (596, 78%). A large number of

patients had a central venous catheter (274, 36%), peripheral vascular access (558, 73%) or a urinary catheter (289, 38%) in place at the time when the BSI was diagnosed. The most commonly reported sources of BSI were intravascular catheters (207, 27%) or a skin or soft tissue infection (152, 20%). Patients with a delay in appropriate therapy of more than one day had stayed in hospital for longer before the BSI (5 days; IQR, 0-17 versus 1 day; IQR, 0-8; p<0.01), and had more often received antibiotic therapy prior to diagnosis (55% versus 37%, p=0.01). They also had a higher proportion of MRSA (61% versus 18%, p<0.01).

In the *E. coli* cohort, the median age was 73 (IQR, 60-81). Ninety-five of 1,028 (9%) patients had G3CREC BSI. The majority was female (575, 56%). Eight hundred forty-six patients (82%) were emergency admissions and a large number had a peripheral vascular access (797, 78%) or a urinary catheter (420, 41%) at the time the diagnostic blood culture was taken. The most common source of BSI was the urinary tract (148, 14%). Similar to the patients in the *S. aureus* cohort, patients with a delay in appropriate therapy of more than one day also had had a longer admission period before the BSI (1 day; IQR, 0-9 versus 0 days; IQR, 0-5; p<0.01), more often had received antibiotic therapy prior to the bacteraemic episode (97, 49% versus 298, 36%, p=0.05) and the proportion of *E. coli* isolates expressing third-generation cephalosporin resistance was higher (22% versus 6%, p<0.01; Table 1)

## Appropriate therapy

Five hundred fifty-five of 763 (73%) patients with *S. aureus* BSI received appropriate therapy within the first day after the index blood culture, after two days this number increased to 623 (82%) patients. Altogether, 697/763 (91%) patients with *S. aureus* BSI received appropriate therapy within 14 days.

For patients with MRSA and MSSA BSI the time to appropriate therapy differed significantly (log-rank test, p<0.01). Figure 1A shows the difference in the first 72 hours, based on 569/763 patients for whom exact data on the timing of blood culture and therapy were available. The median delay was 4 hours (IQR, 1-24) for MSSA patients versus 48 hours (IQR, 12-96) for MRSA patients (Figure 1A; log-rank test, p<0.01). Overall, 96% ($CI_{95}$, 94-97%) of the patients with MSSA BSI received appropriate therapy compared to 82% ($CI_{95}$, 77-87%) of the MRSA patients ($X^2$, p<0.01).

**Table 1.** Characteristics of patients in the *S. aureus* and *E. coli* cohort

| | Characteristics of patients with *S. aureus* BSI and delay in appropriate therapy for | | | | | Characteristics of patients with *E. coli BSI* and delay in appropriate therapy for | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | <2 days | 2-3 days | | > 3 days | | <2 days | 2-3 days | | > 3 days | |
| | Patients (%) N=555 | Patients (%) N=108 | p† | Patients (%) N=100 | p† | Patients (%) N=830 | Patients (%) N=103 | p† | Patients (%) N=95 | p† |
| Female | 215 (39) | 47 (44) | 0.35 | 35 (35) | 0.48 | 470 (57) | 52 (50) | 0.24 | 53 (56) | 0.88 |
| Age* | 67 (55-76) | 67 (56-77) | 0.64 | 73 (63-79) | <0.01 | 73 (60-81) | 77 (64-84) | <0.01 | 73 (60-81) | 0.99 |
| Emergency admission | 434 (78) | 85 (79) | 0.91 | 77 (77) | 0.79 | 691 (83) | 88 (85) | 0.81 | 67 (71) | 0.01 |
| Transfer from another institution# | 72 (15) | 17 (18) | 0.53 | 15 (18) | 0.56 | 101 (14) | 21 (23) | 0.03 | 20 (24) | 0.02 |
| >2 hospital stays in previous year# | 123 (26) | 31 (33) | 0.20 | 30 (36) | 0.07 | 158 (22) | 27 (30) | 0.11 | 30 (36) | 0.01 |
| Positive blood culture at ICU | 70 (13) | 9 (8) | 0.21 | 14 (14) | 0.70 | 58 (7) | 4 (4) | 0.23 | 6 (6) | 0.81 |
| Length of stay before BSI* | 1 (0-8) | 6 (1-14.5) | <0.01 | 4 (0-17.5) | <0.01 | 0 (0-5) | 1 (0-10) | <0.01 | 1 (0-8) | <0.01 |
| Surgery before BSI | 109 (20) | 28 (26) | 0.14 | 21 (21) | 0.75 | 120 (14) | 16 (16) | 0.77 | 14 (15) | 0.94 |
| Prior receipt of antibiotic therapy | 205 (37) | 54 (50) | 0.01 | 60 (60) | <0.01 | 298 (36) | 47 (46) | 0.05 | 50 (53) | <0.01 |
| **CCI** | | | | | | | | | | |
| Charlson score* | 2 (1-4) | 3 (1-5) | <0.01 | 2 (1-4) | 0.10 | 2 (1-3) | 2 (1-4) | 0.15 | 2 (1-4) | 0.05 |
| Myocardial infarct | 61 (11) | 16 (15) | 0.26 | 13 (13) | 0.56 | 76 (9) | 10 (10) | 0.86 | 9 (9) | 0.92 |
| Congestive heart failure | 90 (16) | 26 (24) | 0.05 | 16 (16) | 0.96 | 117 (14) | 16 (16) | 0.69 | 10 (11) | 0.34 |
| Cerebrovascular disease | 61 (11) | 13 (12) | 0.75 | 15 (15) | 0.25 | 81 (10) | 13 (13) | 0.36 | 12 (13) | 0.38 |
| Chronic pulmonary disease | 73 (13) | 15 (14) | 0.84 | 7 (7) | 0.08 | 81 (10) | 15 (15) | 0.13 | 9 (9) | 0.93 |
| Mild liver disease | 23 (4) | 4 (4) | 0.83 | 2 (2) | 0.30 | 33 (4) | 1 (1) | 0.12 | 3 (3) | 0.7 |
| Severe liver disease | 32 (6) | 6 (6) | 0.93 | 4 (4) | 0.48 | 32 (4) | 5 (5) | 0.62 | 4 (4) | 0.87 |
| Severe renal disease | 125 (23) | 29 (27) | 0.33 | 26 (26) | 0.45 | 135 (16) | 23 (22) | 0.12 | 15 (16) | 0.91 |

**Table 1.** Continued

| | Characteristics of patients with *S. aureus* BSI and delay in appropriate therapy for | | | | | Characteristics of patients with *E. coli BSI* and delay in appropriate therapy for | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | <2 days | 2-3 days | | > 3 days | | <2 days | 2-3 days | | > 3 days | |
| | Patients (%) N=555 | Patients (%) N=108 | p† | Patients (%) N=100 | p† | Patients (%) N=830 | Patients (%) N=103 | p† | Patients (%) N=95 | p† |
| Peripheral vascular disease | 55 (10) | 15 (14) | 0.22 | 13 (13) | 0.35 | 61 (7) | 12 (12) | 0.13 | 13 (14) | 0.03 |
| Connective tissue disease | 22 (4) | 9 (8) | 0.05 | 1 (1) | 0.14 | 37 (4) | 4 (4) | 0.79 | 0 (0) | 0.04 |
| Peptic ulcer | 26 (5) | 5 (5) | 0.98 | 6 (6) | 0.57 | 38 (5) | 5 (5) | 0.9 | 5 (5) | 0.76 |
| Diabetes | 122 (22) | 30 (28) | 0.19 | 27 (27) | 0.27 | 185 (22) | 19 (18) | 0.37 | 28 (29) | 0.12 |
| Diabetes with end organ damage | 46 (8) | 7 (6) | 0.53 | 7 (7) | 0.66 | 38 (5) | 8 (8) | 0.16 | 6 (6) | 0.45 |
| Hemi/ paraplegia | 21 (4) | 8 (7) | 0.09 | 10 (10) | 0.01 | 27 (3) | 5 (5) | 0.4 | 5 (5) | 0.31 |
| Cancer/leukaemia | 92 (17) | 22 (20) | 0.34 | 17 (17) | 0.92 | 179 (22) | 23 (22) | 0.86 | 28 (29) | 0.08 |
| Metastatic solid tumour | 34 (6) | 12 (11) | 0.06 | 8 (8) | 0.48 | 59 (7) | 8 (8) | 0.81 | 8 (8) | 0.64 |
| Dementia | 21 (4) | 3 (3) | 0.61 | 10 (10) | 0.01 | 59 (7) | 9 (9) | 0.55 | 7 (7) | 0.93 |
| **Indwelling devices on enrolment** | | | | | | | | | | |
| Intubation | 52 (9) | 6 (6) | 0.20 | 5 (5) | 0.15 | 35 (4) | 2 (2) | 0.26 | 6 (6) | 0.35 |
| Central venous catheter | 195 (35) | 40 (38) | 0.56 | 39 (39) | 0.46 | 135 (16) | 12 (12) | 0.23 | 20 (21) | 0.24 |
| Arterial vascular access | 67 (12) | 11 (10) | 0.58 | 14 (14) | 0.59 | 49 (6) | 4 (4) | 0.66 | 7 (7) | 0.81 |
| Peripheral vascular access | 404 (73) | 80 (75) | 0.40 | 74 (75) | 0.37 | 640 (77) | 81 (79) | 0.42 | 76 (80) | 0.75 |
| Urinary catheter | 196 (35) | 46 (43) | <0.01 | 47 (47) | 0.03 | 341 (41) | 37 (36) | 0.14 | 42 (44) | 0.8 |
| Tracheostomy | 16 (3) | 5 (5) | 0.34 | 5 (5) | 0.27 | 8 (1) | 2 (2) | 0.36 | 3 (3) | 0.06 |
| Nasogastric tube | 75 (14) | 14 (13) | 0.90 | 19 (19) | 0.33 | 56 (7) | 8 (8) | 0.87 | 10 (11) | 0.38 |
| Wound drainage tube | 43 (8) | 8 (7) | 0.90 | 11 (11) | 0.28 | 37 (4) | 2 (2) | 0.23 | 7 (7) | 0.21 |
| **Characteristics of the BSI** | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Hospital acquired BSI (<48 h) | 254 (46) | 76 (70) | <0.01 | 56 (56) | 0.06 | 243 (29) | 42 (41) | 0.02 | 40 (42) | 0.01 |
| Methicillin resistance | 101 (18) | 56 (52) | <0.01 | 71 (71) | <0.01 | na | na | | na | |
| 3rd gen. cephalosporin resistance | na | na | | na | | 51 (6) | 21 (20) | <0.01 | 23 (24) | <0.01 |
| **Source of the BSI** | | | | | | | | | | |
| Bone/ joint | 22 (4) | 3 (3) | | 3 (3) | | 4 (0) | 0 | | 0 | |
| CNS foci | 11 (2) | 0 | | 0 | | 0 | 0 | | 0 | |
| Intervention | 7 (1) | 0 | | 1 (1) | | 21 (3) | 0 | | 2 (2) | |
| Ear-nose-throat | 8 (1) | 2 (2) | | 1 (1) | | 0 | 0 | | 0 | |
| Intra-abdominal | 11 (2) | 2 (2) | | 2 (2) | | 126 (15) | 14 (14) | | 18 (19) | |
| Intravascular | 158 (29) | 20 (19) | | 29 (29) | | 23 (3) | 1 (1) | | 4 (4) | |
| Lower respiratory tract | 57 (10) | 7 (6) | | 9 (9) | | 20 (2) | 3 (3) | | 3 (3) | |
| Skin/ Soft-tissue | 113 (21) | 24 (22) | | 15 (15) | | 16 (2) | 2 (2) | | 3 (3) | |
| Urinary-genital | 16 (3) | 5 (5) | | 6 (6) | | 445 (54) | 57 (56) | | 36 (38) | |
| Unknown | 147 (27) | 45 (42) | | 34 (34) | | 175 (21) | 26 (25) | | 29 (31) | |
| **Outcome** | | | | | | | | | | |
| Death within 30 days after BSI^ | 119 (22) | 19 (18) | 0.64 | 34 (35) | 0.02 | 116 (15) | 8 (8) | 0.12 | 27 (30) | <0.01 |

† P-value derived from the Chi-square or Wilcoxon rank sum test comparing characteristics of the patients in the indicated column with those in the '<2 days' column.
* Median and IQR
# Based on 470, 95, 84 *S. aureus* patients and 706, 90, 84 *E. coli* patients, respectively.
^ Based on 532, 104, 97 *S. aureus* patients and 789, 100, 90 *E. coli* patients, respectively.
na, not applicable

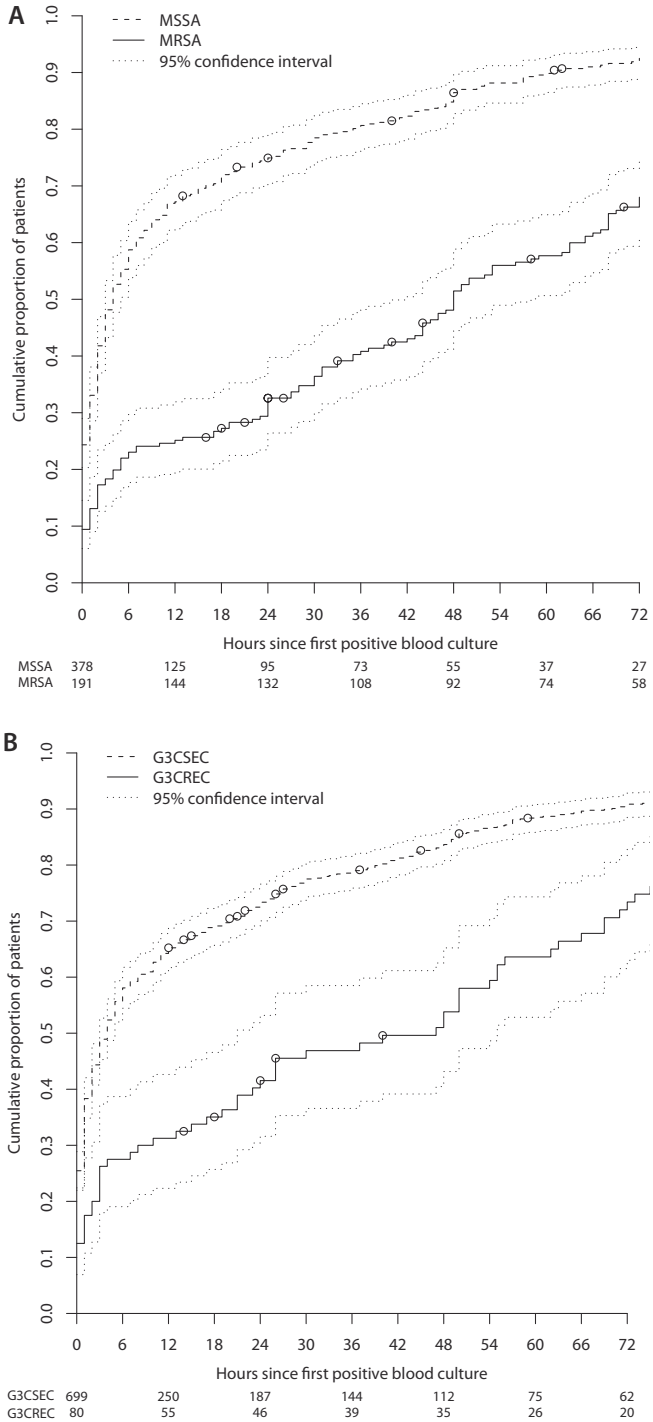



**Figure 1.** Kaplan-Meier curves with time to appropriate therapy for patients with MSSA or MRSA BSI (A) and SEC or G3CREC BSI (B). Open circles refer to censored observations (non-survivors).

Eight hundred-thirty of 1,028 (81%) patients with an *E. coli* BSI received appropriate therapy within the first day after the blood culture, after two days this increased to 909 (88%) patients. Overall, 957/1,028 (93%) of the patients with *E. coli* BSI received appropriate therapy within 9 days. In this cohort, presence of third-generation cephalosporin resistance increased the time to appropriate therapy as well (log-rank test, p<0.01). Based on 779/1,028 patients with data about the exact timing of blood culture and therapy, the median delay for patients with SEC BSI was 4 hours (IQR, 0-26) versus 40 hours (IQR, 3-73) for the G3CREC patients (Figure 1B; log-rank test, p<0.01). Overall, 94% ($CI_{95}$, 92-95%) of the patients with SEC BSIs received appropriate therapy compared to 85% ($CI_{95}$, 78-92%) of the G3CREC patients ($X^2$, p<0.01).

## Thirty-day mortality

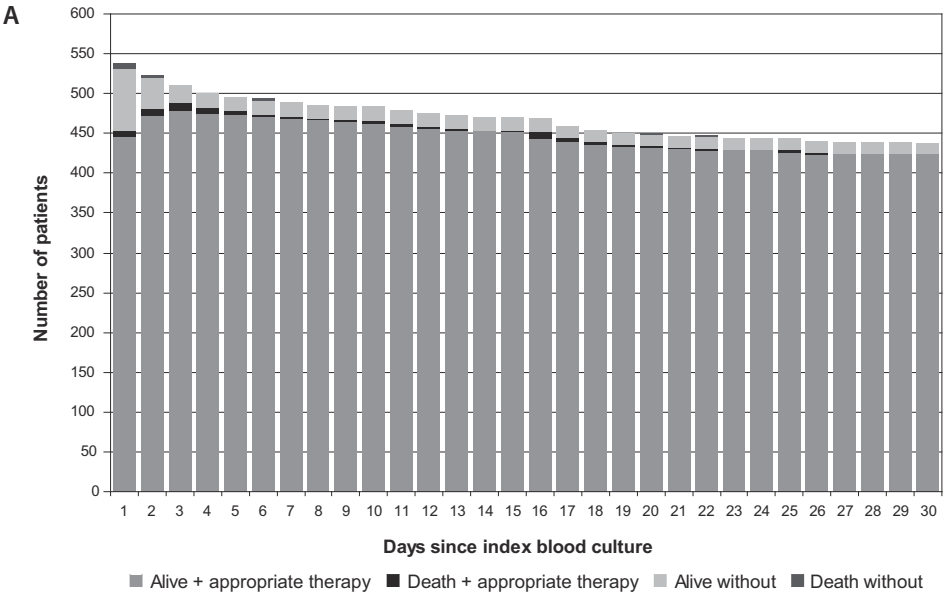
In the *S. aureus* cohort, 30/763 (4%) patients were lost-to-follow-up after being discharged alive. From the remaining 733 patients with *S. aureus* BSI 172 (23%) died within 30 days after the index blood culture. In the group of patients receiving appropriate therapy, 124 of 669 (22%) patients died, compared to 27/64 (42%) patients without appropriate therapy. For patients with MSSA BSI and appropriate therapy, 92/486 (19%) died after a median of 7 days, while 10/23 (43%) without appropriate therapy died after a median of three days. In the MRSA group, 53/183 (29%) patients with appropriate therapy died after a median of 9 days, and 17/41 (41%) patients without appropriate therapy died after a median of two days. In Figure 2, mortality rates for day one to 30 are shown. Most patients without appropriate therapy died in the first days after the index blood culture, when the proportion of patients without appropriate therapy is largest. As a consequence, the incidence of mortality per day is only slightly larger for patient without appropriate therapy versus those with appropriate therapy, especially in the MRSA cohort.

In the *E. coli* cohort, 49/1,028 (5%) were lost-to-follow-up after being discharged alive. From the remaining patients 151/979 (15%) died within 30 days. For patients receiving appropriate therapy, 126/912 (14%) died, compared to 25/67 (37%) without appropriate therapy. For patients with SEC BSI and appropriate therapy, 103/833 (12%) died after a median of 30 days and 18/53 (34%) without appropriate therapy after a median of 13 days. In the group of patients with G3CREC BSI and appropriate therapy, 23/79 (29%) died after a median of 9 days, while 7/14 (50%) without appropriate therapy died after a median of 8 days. The mortality rate per

day for SEC and G3CREC showed the same pattern as for the MSSA and MRSA cohorts, respectively.

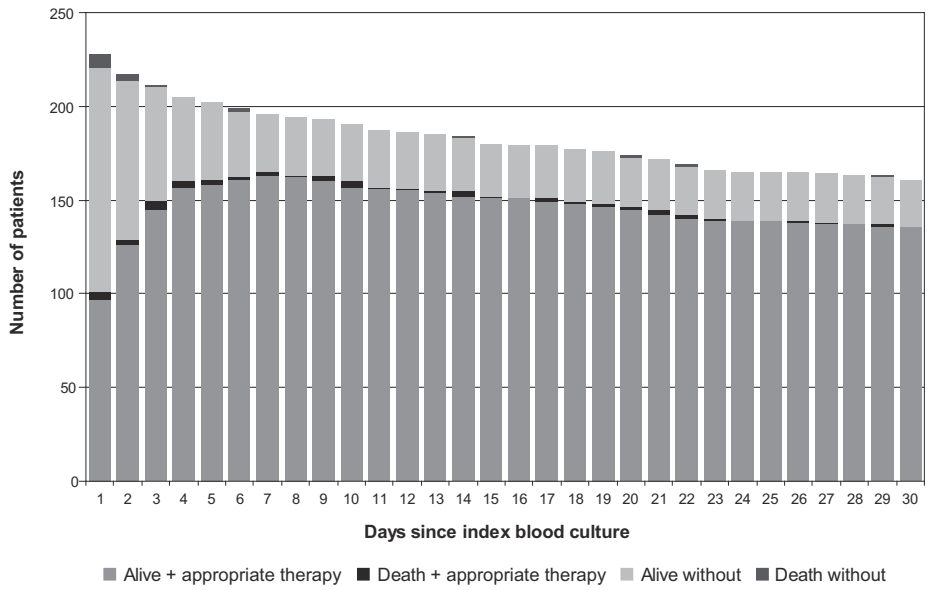
Since methicillin resistance in *S. aureus* BSI and third-generation cephalosporin resistance in *E. coli* BSI were strongly related with delay in appropriate therapy, different survival models were created for patients with MSSA, MRSA, SEC or G3CREC BSI. In addition, cluster effects at hospital level changed the effect estimate more than 5% and therefore hospital was included as stratum variable.

Survival models, adjusted for immortal time bias by including appropriate therapy as a time-dependent variable[5], showed that appropriate therapy decreased 30-day mortality in the group of patients with MSSA BSI (HR, 0.47; $CI_{95}$, 0.23-0.95) and SEC BSI (HR, 0.44; $CI_{95}$, 0.26-0.75). In the MRSA and G3CREC cohorts the HRs for appropriate therapy were close to one and insignificant (MRSA HR, 1.01, $CI_{95}$, 0.56-1.83; G3CREC, HR, 1.20, $CI_{95}$, 0.48-2.99). Multivariate analyses gave similar results as the univariate analysis, appropriate therapy significantly reduced 30-day mortality in the MSSA (HR, 0.34; $CI_{95}$, 0.17-0.67) and SEC cohort (HR, 0.45; $CI_{95}$, 0.27-0.77) (Table 2).



| Mortality rate per patient day | | | | | | | |
|---|---|---|---|---|---|---|---|
| Day | 1 | 2 | 3 | 4-5 | 6-10 | 11-20 | 21-30 |
| Appropriate therapy | 0.02 | 0.02 | 0.02 | 0.01 | 0.01 | 0.01 | 0.00 |
| Without appropriate therapy | 0.04 | 0.05 | 0.09 | 0.00 | 0.00 | 0.01 | 0.01 |
| p-value | 0.53 | 0.43 | 0.17 | 0.99 | 0.99 | 0.59 | 0.58 |

**B**




| Mortality rate per patient day | | | | | | | |
|---|---|---|---|---|---|---|---|
| Day | 1 | 2 | 3 | 4-5 | 6-10 | 11-20 | 21-30 |
| Appropriate therapy | 0.04 | 0.02 | 0.03 | 0.02 | 0.01 | 0.01 | 0.01 |
| Without appropriate therapy | 0.06 | 0.03 | 0.02 | 0.00 | 0.01 | 0.01 | 0.01 |
| p-value | 0.82 | 0.93 | 0.88 | 0.74 | 0.99 | 0.99 | 0.99 |

**Figure 2.** Mortality rate for each day since the index blood culture, for patients with and without appropriate therapy at that moment in time, plotted separately for patients with MSSA BSI (A) or MRSA BSI (B).

**Table 2.** Hazard ratios for the impact of appropriate therapy received within two days after infection (empirical) or independent of timing, on 30-day mortality for patients with MSSA and MRSA, SEC and G3CREC BSI.[a]

| Effect measure | Hazard ratio for effect measure ($CI_{95}$) | | Confounders |
|---|---|---|---|
| | Univariate | Multivariate | |
| *MSSA* | N=535 | | |
| Empirical appropriate therapy | 0.58 (0.32-1.05) | 0.56 (0.30-1.04) | Age, urinary catheter, intubation |
| Appropriate therapy | 0.47 (0.23-0.95) | 0.34 (0.17-0.67) | |
| *MRSA* | N=226 | | |
| Empirical appropriate therapy | 1.01 (0.61-1.68) | 1.12 (0.65-1.94) | Urinary catheter, intubation, CCI |
| Appropriate therapy | 1.01 (0.56-1.83) | 1.05 (0.57-1.92) | |
| *SEC* | N=933 | | |
| Empirical appropriate therapy | 0.47 (0.29-0.75) | 0.47 (0.28-0.77) | Intubation, CCI, LOS before infection |
| Appropriate therapy | 0.44 (0.26-0.75) | 0.45 (0.27-0.77) | |
| *G3CREC* | N=95 | | |
| Empirical appropriate therapy | 1.59 (0.65-3.85) | 1.24 (0.46-3.31) | Age, intubation, CCI, severe liver disease, interaction age and Charlson score |
| Appropriate therapy | 1.20 (0.48-2.99) | 0.94 (0.29-3.10) | |

N, number of patients included in the analysis
[a] Based on Cox's regression analysis with appropriate therapy included as time-dependent variable

# Discussion

In clinical settings, where an experimental study is prohibited for ethical reasons, an observational study is the only way to quantify the impact of appropriate therapy. As a consequence, acknowledgement of possible confounding and bias is essential. This study confirmed that patients with MRSA or G3CREC infections experienced significantly longer waiting times till appropriate therapy was administered compared to patients infected by susceptible bacteria. Therefore, we analysed the impact of appropriate therapy separately for MSSA, MRSA, SEC and G3CREC BSIs. Taking into account timing of therapy, appropriate therapy was associated with a significantly improved survival for patients with MSSA BSI (HR 0.47; $CI_{95}$, 0.23-0.95) or SEC BSI (HR, 0.44; $CI_{95}$, 0.26-0.75). For the two resistant cohorts an impact of appropriate therapy could not be ascertained.

This study is unique in several ways. First of all, the presented data included

patients from thirteen different tertiary care centres spread over Europe. Variation in resistance endemicity, for MRSA ranging from 7% to 56% and for G3CREC from 4% to 24% [6], suggests that the outcomes from this study are representative for the situation in Western and Central Europe. Second, mortality was not measured at discharge, but after a predefined interval of 30 days after ascertainment of infection, including follow-up beyond hospital discharge, preventing informative censoring. Third, survival analysis was used, with appropriate therapy included as a time-dependent variable. In this way, the spurious positive association between longer pre-treatment survival, a higher likelihood of receiving appropriate therapy and longer post-treatment survival was eliminated from the analysis.[5] This was especially important in the cohorts of patients with infections due to resistant micro-organisms, because time from blood culture to appropriate therapy was longer in these groups. Finally, the appropriateness of therapy was not based on subjective judgments, but based on the objective measurement of *in vitro* susceptibility and accepted rules of beta-lactam class resistance of the isolated pathogen.

Other studies confirm that patients with infections complicated by resistance are faced with prolonged delays in administration of appropriate therapy.[8,9] However, so far there is no consensus about the clinical effectiveness of therapy. Most studies included inappropriate therapy as a risk factor for mortality, whereby some found significant impacts ranging from an odds ratio (OR) of 1.09 ($CI_{95}$, 1.01-1.14)[10] to 18.0 ($CI_{95}$, 2.8-114.5)[11], others could not confirm higher mortality rates.[8,12-14] These differences can be explained by inclusion of varying populations, whereby the consequences of inappropriate therapy tend to be pronounced for severely ill patients.[9,11,15-18] Part of these differences may also be related to varying degrees of bias; only few of the above referenced studies included follow-up beyond hospital discharge[9,13,15], just one analysed the impact of therapy separately for resistant and susceptible cases[19], and none of previously published studies analysed therapy as time-dependent factor in survival analysis.

Although the association between appropriate therapy and improved survival may seem intuitive, there are several, possible explanations why we did not find the same association for patients with resistant infections. A limitation of this study was lack of data about dosing and serum levels, especially for vancomycin, but we accepted this trade off as a concession to this international multi-centre study design. An alternative, methodological explanation for the absence of an effect in the resistant cohorts may be a lack of power; i.e. the number of cases might have been too low to be able to detect a weak association. This would be true

if the benefit of appropriate therapy over inactive therapy was small, as may be the case for MRSA infections. Vancomycin, the most important active antibiotic for MRSA patients, may be inferior to beta-lactam antibiotics active against MSSA.[20,21] At the other end, treatment of MRSA with beta-lactam antibiotics, not deemed effective, may still have some residual effect.[22] Finally, timing of therapy could have influenced the potentially beneficial effect. Previous studies have shown that most fatalities among bacteraemic patients occur within the first two days[23,24], indicating that there is a limited window of opportunity for therapy. Patients initially treated inappropriately are faced with an increasing microbial burden, increasing inflammatory cellular dysfunction and tissue injury, which can lead to shock and once irreversible organ injury is present, death is inevitable.[23,25] In this study less than 50% of the patients in the resistant cohorts received appropriate therapy within the first two days and most patients dying without appropriate therapy died in this period. This suggests that for those patients who could benefit most from appropriate therapy, initiation of appropriate therapy was too late.

To conclude, most patients suffering from susceptible *S. aureus* and *E. coli* BSIs are treated timely and effectively in European hospitals. For patients affected by resistant infections, the often delayed appropriate therapy was not improving survival. This study stresses the importance of early, adequate empirical treatment of septicaemic patients. And the results underline the value of local surveillance of antimicrobial resistance in combination with demographic and clinical data in order to improve the choice of antibiotic compounds in the individual patient.

## Acknowledgements

This work was funded by DG-Sanco (grant number 2005203), RIVM, and UMCG.
We would like to acknowledge Anja Haenen, Jos Monen and Ineke Roede from RIVM for their assistance in assessment of appropriateness of therapy and Birgit van Benthem and Hendriek Boshuizen from RIVM for their valuable epidemiological and statistical advice.

# Reference List


1. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. (2003) Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteraemia: a meta-analysis. Clin Infect Dis 36: 53-9.
2. Schwaber MJ, Navon-Venezia S, Kaye KS, Ben-Ami R, Schwartz D, Carmeli Y. (2006) Clinical and economic impact of bacteraemia with extended- spectrum-beta-lactamase-producing Enterobacteriaceae. Antimicrob Agents Chemother 50: 1257-62.
3. De Kraker MEA , Wolkewitz M, Davey PG, Koller W, Berger J, Nagler J, *et al*. (2011) Clinical impact of antimicrobial resistance in European hospitals: Excess mortality and length of stay related to methicillin-resistant *Staphylococcus aureus* bloodstream infections. Antimicrob Agents Chemother 55: 1598-1605.
4. De Kraker MEA , Wolkewitz M, Davey PG, Koller W, Berger J, Nagler J, et al. (2011) Burden of antimicrobial resistance in European hospitals: Excess mortality and length of hospital stay associated with bloodstream infections due to *Escherichia coli* resistant to third-generation cephalosporins. J Antimicrob Chemother 66: 398-407.
5. Levesque LE, Hanley JA, Kezouh A, Suissa S. (2010) Problem of immortal time bias in cohort studies: example using statins for preventing progression of diabetes. BMJ 340: b5087.
6. EARSS-MT, Advisory Board, and and National Representatives (2009) EARSS annual report 2008.On-going surveillance of *S. pneumoniae, S. aureus, E. coli, E. faecium, E. faecalis, K. pneumoniae, P. aeruginosa*. Available: http://www.ecdc.europa.eu/en /activities/ surveillance/ EARS-Net/Documents/2008_EARSS_Annual_Report.pdf. Accessed April 2012.
7. Charlson ME, Pompei P, Ales KL, MacKenzie CR. (1987) A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis 40: 373-83.
8. Ammerlaan H, Seifert H, Harbarth S, Brun-Buisson C, Torres A, Antonelli M, *et al*. (2009) Adequacy of antimicrobial treatment and outcome of *Staphylococcus aureus* bacteraemia in 9 Western European countries. Clin Infect Dis 49: 997-1005.
9. Paul M, Kariv G, Goldberg E, Raskin M, Shaked H, Hazzan R, *et al.* (2010) Importance of appropriate empirical antibiotic therapy for methicillin-resistant *Staphylococcus aureus* bacteraemia. J Antimicrob Chemother 65: 2658-65.
10. Garnacho-Montero J, Aldabo-Pallas T, Garnacho-Montero C, Cayuela A, Jimenez R, Barroso S, *et al.* (2006) Timing of adequate antibiotic therapy is a greater determinant of outcome than are TNF and IL-10 polymorphisms in patients with sepsis. Crit Care 10: R111.
11. Lin MY, Weinstein RA, Hota B. (2008) Delay of active antimicrobial therapy and mortality among patients with bacteraemia: impact of severe neutropenia. Antimicrob Agents Chemother 52: 3188-94.
12. Scarsi KK, Feinglass JM, Scheetz MH, Postelnick MJ, Bolon MK, Noskin GA. (2006) Impact of inactive empiric antimicrobial therapy on inpatient mortality and length of stay. Antimicrob Agents Chemother 50: 3355-60.
13. Fang CT, Shau WY, Hsueh PR, Chen YC, Wang JT, Hung CC, *et al*. (2006) Early empirical glycopeptide therapy for patients with methicillin-resistant *Staphylococcus aureus* bacteraemia: impact on the outcome. J Antimicrob Chemother 57: 511-9.

14. Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, *et al*. (2004) Bloodstream infections due to extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. Antimicrob Agents Chemother 48: 4574-81.
15. Garnacho-Montero J, Garcia-Garmendia JL, Barrero-Almodovar A, Jimenez-Jimenez FJ, Perez-Paredes C, Ortiz-Leyba C. (2003) Impact of adequate empirical antibiotic therapy on the outcome of patients admitted to the intensive care unit with sepsis. Crit Care Med 31: 2742-51.
16. Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, *et al*. (2005) Risk factors for antimicrobial resistance and influence of resistance on mortality in patients with bloodstream infection caused by *Pseudomonas aeruginosa*. Microb Drug Resist 11: 68-74.
17. Cisneros JM, Reyes MJ, Pachon J, Becerril B, Caballero FJ, Garcia-Garmendia JL, *et al.* (1996) Bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical findings, and prognostic features. Clin Infect Dis 22: 1026-32.
18. Kang CI, Kim SH, Park WB, Lee KD, Kim HB, Kim EC, *et al.* (2005) Bloodstream infections caused by antibiotic-resistant gram-negative bacilli: risk factors for mortality and impact of inappropriate initial antimicrobial therapy on outcome. Antimicrob Agents Chemother 49: 760-6.
19. Khatib R, Saeed S, Sharma M, Riederer K, Fakih MG, Johnson LB. (2006) Impact of initial antibiotic choice and delayed appropriate treatment on the outcome of *Staphylococcus aureus* bacteraemia. Eur J Clin Microbiol Infect Dis 25: 181-5.
20. Gould IM. (2011) Clinical activity of anti-gram-positive agents against methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 66 Suppl 4: iv17-iv21.
21. Deresinski S. (2009) Antibiotic therapy of vascular catheter-related bloodstream infections: is vancomycin the optimal choice for *Staphylococcus aureus* infections? Int J Antimicrob Agents 34 Suppl 4: S43-6.
22. Guignard B, Entenza JM, Moreillon P. (2005) Beta-lactams against methicillin-resistant *Staphylococcus aureus*. Curr Opin Pharmacol 5: 479-89.
23. Kreger BE, Craven DE, McCabe WR. (1980) Gram-negative bacteraemia. IV. Re-evaluation of clinical features and treatment in 612 patients. Am J Med 68: 344-55.
24. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, *et al*. (2006 ) Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 34: 1589-96.
25. Kumar A. (2010) Early antimicrobial therapy in severe sepsis and septic shock. Curr Infect Dis Rep 12: 336-44.