Supplemental file

Preventive antibiotic therapy in subgroups of acute stroke patients: an individual patient meta-analysis

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### Supplemental Table 1. Search Strategy

| Searches                                                                                                                                                                                                                                                                                                                                 | Results  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| 1  cerebrovascular disorders/ or exp basal ganglia cerebrovascular disease/ or exp brain ischemia/ or exp carotid artery diseases/ or exp cerebrovascular trauma/ or exp intracranial arterial diseases/ or exp intracranial arteriovenous malformations/ or exp 'intracranial embolism and thrombosis'/ or exp intracranial hemorrhages/ or stroke/ or exp brain infarction/ or vasospasm, intracranial/ or vertebral artery dissection/ | 364994  |
| 2  (stroke$ or poststroke$ or cva$ or cerebrovascular$ or cerebral vascular$).tw.                                                                                                                                                                                                 | 313781  |
| 3  ((cerebral or cerebellar or brain$ or vertebrobasilar) adj5 (infarct$ or isch?emi$ or thrombo$ or apoplexy or emboli$)).tw.                                                                                                                                                     | 94335   |
| 4  ((cerebral or intracerebral or intracranial or brain or cerebellar or subarachnoid) adj5 (haemorrhage or hemorrhage or haematoma or hematoma or bleeding or aneurysm)).tw.                                                                                       | 72576   |
| 5  1 or 2 or 3 or 4                                                                                                                                                                                                                                                      | 558713  |
| 6  Antibiotic Prophylaxis/                                                                                                                                                                                                                                               | 14374   |
| 7  exp Anti-Bacterial Agents/                                                                                                                                                                                                                                           | 747215  |
| 8  (antibiotic$ or anti-bacterial or anti bacterial or bacteriocid$ or anti-mycobacterial or anti mycobacterial or anti-myocobacterial or anti-infect$ or anti infect$).tw.                                                                 | 427649  |
| 9  (amoxicillin or amphotericin b or ampicillin or calcimycin or cephalosporin$ or cephalothin or cephaparvin$ or chloramphenicol or dactinomycin or doxycycline or erythromycin or fluoroquinolone$s or gentamicin$ or kanamycin or minocycline or neomycin or oxytetracycline or penicillin or streptomycin or tetracycline or vancomycin$).tw. | 270851  |
| 10 7 or 8 or 9                                                                                                                                                                                                                                                          | 1007805 |
| 11 exp infection/ or exp bacterial infections/ or exp infection control/ or exp fever/ or exp inflammation/                                                                                                                                                              | 2946884 |
| 12 (infection$ or sepsis or septicaemia or septicemia or pneumonia or bacteremia or bacteraemia or inflammation or fever or blood poisoning).tw.                                                                                                                                  | 2138905 |
| 13 11 or 12                                                                                                                                                                                                                                                               | 3893655 |
| 14 (prophylaxis or prevent$ or premedication or incidence or occurrence).tw.                                                                                                                                                                                               | 2593240 |
| 15 prevention control.fs.                                                                                                                                                                                                                                              | 1336031 |
| 16 15 or 14                                                                                                                                                                                                                                                               | 3433905 |
| 17 10 and 13 and 16                                                                                                                                                                                                                                                     | 115044  |
| 18 6 or 17                                                                                                                                                                                                                                                                | 120953  |
| 19 5 and 18                                                                                                                                                                                                                                                               | 1055    |

| Searches                                                                                                                                                                                                                                                                                                                                 | Results  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| 1  cerebrovascular disease/ or basal ganglion hemorrhage/ or exp brain hematoma/ or exp brain hemorrhage/ or exp brain infarction/ or exp brain ischemia/ or exp carotid artery disease/ or exp cerebral artery disease/ or cerebrovascular accident/ or exp cerebrovascular malformation/ or exp intracranial aneurysm/ or exp occlusive cerebrovascular disease/ or stroke/ | 782967  |
| 2  stroke unit/ or stroke patient/                                                                                                                                                                                                                                | 37529   |
| 3  (stroke$ or poststroke$ or cva$ or cerebrovascular$ or cerebral vascular$).tw.                                                                                                                                                                                  | 507145  |
| 4  ((cerebral or cerebellar or brain$ or vertebrobasilar) adj5 (infarct$ or isch?emi$ or thrombo$ or apoplexy or emboli$)).tw.                                                                                                                                          | 137661  |
| 5  ((cerebral or intracerebral or intracranial or brain or cerebellar or subarachnoid) adj5 (haemorrhage or hemorrhage or haematoma or hematoma or bleeding or aneurysm)).tw.                                                                                  | 109930  |
| 6  1 or 2 or 3 or 4 or 5                                                                                                                                                                                                                                           | 939203  |
| 7  antibiotic prophylaxis/                                                                                                                                                                                                                                           | 33976   |
| 8  exp antibiotic agent/                                                                                                                                                                                                                                           | 1681145 |
| 9  (antibiotic$ or anti-bacterial or anti bacterial or bacteriocid$ or anti-mycobacterial or anti mycobacterial or anti-myocobacterial or anti-infect$ or anti infect$).tw.                                                                 | 615316  |
| 10  (amoxicillin or amphotericin b or ampicillin or calcimycin or cephalosporin$ or cephalothin or cephaparvin$ or chloramphenicol or dactinomycin or doxycycline or erythromycin or fluoroquinolone$s or gentamicin$ or kanamycin or minocycline or neomycin or oxytetracycline or penicillin or streptomycin or tetracycline or vancomycin$).tw. | 378306  |
| 11 8 or 9 or 10                                                                                                                                                                                                                                                        | 1955352 |
| 12 exp infection/ or infection control/ or infection risk/ or fever/ or exp inflammation/                                                                                                                                                              | 6504644 |
|   | Search                                                                 | Results |
|---|------------------------------------------------------------------------|---------|
| 1 | ((stroke$ or poststroke$ or cva$ or cerebrovascular* or cerebral vascular)):TI,AB,KY" | 20732   |
| 2 | (((cerebral or cerebellar or brain$ or vertebobasilar) adj5 (infarct$ or isch?emi$: or thrombo$: or apoplexy or emboli$)):TI,AB,KY | 142     |
| 3 | (((cerebral or intracerebral or intracranial or brain or cerebellar or subarachnoid) adj5 (haemorrhage or hemorrhage or haematoma or hematoma or bleeding or aneurysm)):TI,AB,KY | 9786    |
| 4 | 1 OR 2 OR 3                                                           | 28984   |
| 5 | ((antibiotic$ or anti-bacterial or anti bacterial or antibacterial or bacteriocid$: or antimycobacterial or anti mycobacterial or anti mycobacterial or anti infect$: or anti infect$)):TI,AB,KY | 13632   |
| 6 | ((amoxicillin or amphotericin b or ampicillin or calcimycin or cephalosporin$ or cephalothin or cephaparin$: or chloramphenicol or dactinomycin or doxycycline or erythromycin or fluoroquinolone$ or gentamicin$: or kanamycin or minocycline or neomycin or oxytetracycline or penicillin or streptomycin or tetracycline or vancomycine)):TI,AB,KY | 20260   |
| 7 | 7 OR 5 OR 6                                                           | 29471   |
| 8 | ((infection$: or sepsis or septicaemia or septicemia or pneumonia or bacteremia or bacteremia or inflammation or fever or blood poisoning)):TI,AB,KY | 83126   |
| 9 | ((prophyla$: or prevent$: or premedicat$: or incidence or occurrence)):TI,AB,KY" | 132756  |
| 10| 4 and 7 and 8 and 9                                                   | 7       |
### Supplemental table 2. Excluded studies based on fulltext screening

| Author, year                        | Reason for exclusion                                           |
|-------------------------------------|----------------------------------------------------------------|
| Nyren et al, 1981 (23)              | Solely patients with indwelling catheters                      |
| Maijkowski et al, 1982 (24)         | Randomization procedure unclear                                |
| Fagan et al, 2010 (25)              | Not a randomized study                                         |
| Switzer et al, 2011 (26)            | Not a randomized study                                         |
| Ulm et al, 2017 (27)                | Treatment with preventive antibiotic therapy was guided by procalcitonin levels |

### Supplemental table 3a. Studies eligible for including but no data obtained

| Author, year                        | Reason for exclusion                                           |
|-------------------------------------|----------------------------------------------------------------|
| Padma Srivastava et al, 2012 (15)   | Study was eligible for inclusion, no response from authors     |
| Schwarz et al, 2007 (6)             | Study was eligible for inclusion, no response from authors     |
| Lampl et al, 2007 (16)              | Study was eligible for inclusion, contact with author: database no longer available, all authors retired or working elsewhere |

### Supplemental table 3b. Excluded studies based on discrepancies in data with original publication

| Author, year                        | Reason for exclusion                                           |
|-------------------------------------|----------------------------------------------------------------|
| DeFalco et al, 1998 (28)            | Received data did not match original publication               |
| Variable                                                                 | Type 1 trial (aimed at prevention of infection) | Type 2 trial (Minocyclin) |
|-------------------------------------------------------------------------|-------------------------------------------------|---------------------------|
| Randomization                                                           | X                                               | X                         |
| Sex                                                                     | X                                               | X                         |
| Age                                                                     | X                                               | X                         |
| Medical history of atrial fibrillation                                  | X                                               | X                         |
| Medical history of chronic obstructive pulmonary disease                | X                                               | X                         |
| Medical history of diabetes                                             | X                                               | X                         |
| Score on modified Rankin Scale before stroke                            | X                                               | X                         |
| Stroke type                                                             | X                                               | X                         |
| Stroke severity at admission on NIHSS                                    | X                                               | X                         |
| Treatment with iv-thrombolysis                                          | X                                               | X                         |
| Use of urinary catheter                                                 | X                                               | X                         |
| Dysphagia                                                               | X                                               | X                         |
| Diagnosis of infection during admission                                 | X                                               | X                         |
| Days to diagnosis of infection                                          | X                                               | X                         |
| Diagnosis of pneumonia during admission                                 | X                                               | X                         |
| Days to diagnosis of pneumonia                                         | X                                               | X                         |
| Days to diagnosis of UTI during admission                               | X                                               | X                         |
| Diagnosis of other infection during admission                           | X                                               | X                         |
| Days to diagnosis of other infection                                    | X                                               | X                         |
| Length of hospital stay                                                 | X                                               | X                         |
| Discharge - score on modified Rankin Scale                              | X                                               | X                         |
| Discharge - unfavorable outcome                                         | X                                               | X                         |
| Discharge - mortality                                                   | X                                               | X                         |
| 3 months – score on modified Rankin Scale                               | X                                               | X                         |
| 3 months - NIHSS                                                        | X                                               | X                         |
| 3 months – unfavorable functional outcome *                             | X                                               | X                         |
| 3 months – Barthel index                                                | X                                               | X                         |
| Therapy with preventive antibiotic therapy: name, class, route and dosage| X                                               | X                         |
| Number of days of treatment with preventive antibiotic therapy          | X                                               | X                         |
| Treatment per protocol                                                  | X                                               | X                         |
| Time to first dose of preventive antibiotic therapy                     | X                                               | X                         |
| Serious adverse events                                                  | X                                               | X                         |

* mRS=3-6; BI <60 or deceased
| Supplemental table 5. Definition of baseline characteristics (when ascertained) |
|--------------------------------------------------|
| **Age** | All trials: age of the patient in years at day of inclusion in the trial |
| **Sex** | All trials: sex of the patient as reported by patient |
| **Medical history of atrial fibrillation** | - Kalra et al: assessed by physician who admitted the patient from medical records, new diagnosis if atrial fibrillation on ECG at admission.  
- Westendorp et al: assessed by physician who admitted the patient from medical records, new diagnosis if atrial fibrillation on ECG at admission.  
- Harms et al: taken from the medical records of the patient  
- Chang et al: by reported history or atrial fibrillation noted during hospitalization/ telemetry  
- Blacker et al: by standard ECG, or documentation in medical record  
- Kohler et al: by standard ECG, or documentation in medical record |
| **Medical history of obstructive pulmonary disease** | - Kalra et al: assessed by physician who admitted the patient from medical records.  
- Westendorp et al: assessed by physician who admitted the patient from medical records.  
- Harms et al: taken from the medical records of the patient  
- Chamorro et al: -  
- Blacker et al: documentation in medical record  
- Kohler et al: documentation in medical record |
| **Medical history of diabetes mellitus** | - Kalra et al: assessed by physician who admitted the patient from medical records.  
- Westendorp et al: assessed by physician who admitted the patient from medical records.  
- Harms et al: taken from the medical records of the patient  
- Chamorro et al: -  
- Chang et al: by reported history  
- Fouda et al: documented history in medical record or reported history by patient or family  
- Blacker et al: new diagnosis if BSL > 11, or documentation in medical record  
- Amiri-Nikpour et al: any drugs used for treating diabetes and medical documents confirming the diagnosis of diabetes. The patient and his/her family members/proxy were also used for confirming the diagnosis in condition it was found to be reliable. In addition, we also routinely checked fasting blood glucose and blood glucose to assess the diabetes status of patients.  
- Kohler et al: new diagnosis if BSL > 11, or documentation in medical record |
| **Pre-stroke disability (mRS)** | - Kalra et al: assessed by physician at admission  
- Westendorp et al: assessed by physician at admission  
- Chang et al: assessed at admission by neurologist  
- Blacker et al: assessed at admission on modified rankin scale |
| **Stroke type** | - Kalra et al: -  
- Westendorp et al: physician’s assessment, always included head-CT-scan, some cases also MRI  
- Harms et al: clinical diagnosis of an acute ischemic stroke in the middle cerebral artery territory and NIHSS>11 between 9 and 36h of symptoms onset (exclusion of ICH by CT)  
- Chamorro et al: brain CT or MRI  
- Chang et al: study was only hemorrhagic stroke/ ICH. Defined by CTH.  
- Fouda et al: CT-scan  
- Blacker et al: CT or MRI  
- Amiri-Nikpour et al: CT-scan  
- Kohler et al: CT or MRI |
| **Stroke severity** | - Kalra et al: assesses on NIHSS by physician  
- Westendorp et al: assessed on NIHSS by resident neurology / neurologist, in most, but not all, centers trained in NIHSS scoring  
- Harms et al: assessed on NIHSS by study physicians (neurologists trained in stroke medicine and NIHSS scoring)  
- Chamorro et al: scored on NIHSS by physician  
- Chang et al: neurologist (myself) on NIHSS; yes trained in NIHSS  
- Fouda et al: co-investigators trained for NIHSS scoring  
- Blacker et al: on NIHSS, usually a neurology trainee, sometimes a consultant neurologist; MOST (not all), had done NIHSS training. Clinicians all had access to written aids to calculate the NIHSS.  
- Amiri-Nikpour et al: assessed on NIHSS by physicians trained for NIHSS assessment  
- Kohler et al: on NIHSS, usually by a neurology trainee, sometimes a consultant neurologist; MOST (not all), had done NIHSS training. Clinicians all had access to written aids to
calculate the NIHSS.

| Intravenous thrombolysis | Kalra et al. - 
Westendorp et al.: Actilyse 1mg/ml according to the weight of a patient 
Harms et al.: Actilyse according to the guidelines (0.9mg/kg bw in max 90mg, 10% Bolus, 90% over 60min; within 3.5 hours) 
Chamorro et al.: no patients received iv-thrombolysis 
Chang et al.: not applicable (solely hemorrhagic stroke included) 
Fouda et al.: not applicable 
Blacker et al.: alteplase, 0.9mg/kg as per standard protocol based on state and national guidelines. 
Amiri-Nikpour et al.: no patients received iv-thrombolysis 
Kohler et al.: alteplase, 0.9mg/kg as per standard protocol based on state and national guidelines. |
|---------------------------|--------------------------------------------------|
| Use of urinary catheter    | Westendorp et al.: use of urinary catheter during (a part of) the admission 
Chang et al.: use of urinary catheter during admission |
| Dysphagia                 | Kalra et al.: inclusion in the trial was based on dysphagia. Dysphagia-trained nursing staff assessed swallowing using the standard bedside swallowing assessment test consisting of measuring levels of consciousness, oromotor function, and consumption of water or food. 
Westendorp et al.: the presence of any type of dysphagia as assessed by a dysphagia trained nurse and/or speech therapist. |
| Diagnosis of infection / pneumonia / UTI / other infection and time-frame in which this was assessed. | Kalra et al.: diagnosis of pneumonia made by the local treating physician during 14 days. Urinary tract infection and other infections were extracted from the serious adverse events registry. 
Westendorp et al.: assessed by physician and assessed by independent panel with infectious diseases specialists with modified CDC criteria during hospital admission. 
Chamorro et al.: infection was defined if temperature >37.5°C in 2 determinations or >37.8°C in a single determination in patients with suggestive symptoms (ie, cough, dyspnea, pleuritic pain, urinary tract symptoms), white blood cell count >11 000/mL or <4000/mL, pulmonary infiltrate on chest x-rays, or cultures positive for a pathogen. Otherwise, temperature >37.8°C was classified as noninfectious hyperthermia. Infection was further classified as early, if it occurred within the first 7 days after stroke, and late, when it supervened between days 8 and 90 after stroke. For the current analysis infection within 7 days was used. 
Harms et al.: modified CDC criteria within 11 days. 
Chang et al.: imaging, leukocytosis, fevers, clinical deterioration during follow-up (until 90 days) 
Blacker et al.: routine clinical diagnosis, rather than strict criteria; eg urine cultures showing white cells and bacteria in associated with appropriate clinical symptoms, or cough and sputum in association with changes seem on chest X-ray to diagnose respiratory infections, or skin redness, warmth and tenderness to diagnose cellulitis or phlebitis within 7 days post stroke. 
Kohler et al.: routine clinical diagnosis, rather than strict criteria; eg urine cultures showing white cells and bacteria in associated with appropriate clinical symptoms, or cough and sputum in association with changes seem on chest X-ray to diagnose respiratory infections, or skin redness, warmth and tenderness to diagnose cellulitis or phlebitis within 7 days post stroke. |
| Time to infection          | Time to diagnosis of infection in days |
| Unfavorable outcome        | Harms et al: mRS was not collected. Definition used: death or BI < 60. 
For other trials: mRS 3-6. |
| Modified Rankin Scale Score| Kalra et al.: assessed by by trial office researchers masked to allocation. 
Westendorp et al.: assessed by structured telephone interview by blinded trained trial team member. 
Chamorro et al.: 
Chang et al.: assessed by neurologist 
Fouda et al.: primary clinical outcome was mRS at 90 days performed by co-investigator trained for mRS scoring. 
Blacker et al.: telephone interview by researchers from another hospital, blinded to the treatment allocation; standardized questionnaire to assess for recurrent vascular events; modified Rankin score to assess outcome; personnel were trained in modified Ranking |
| Preventive antibiotic therapy name | When preventive antibiotic was given to a patient, name of antibiotic |
|-----------------------------------|---------------------------------------------------------------------|
| **Antibiotic therapy class**      | When preventive antibiotic was given to a patient, class of antibiotic. Class of antibiotic: 1=tetracyclin (minocyclin), 2=cefalosporins (ceftriaxone), 3=fluorchinolon (levofloxacine, moxifloxacine) 4= penicillin + macrolide (amoxicillin + clarithromycin) |
| Type of antibiotics               | Kalra et al: treating physician was able to choose the type of antibiotic, but a recommendation was made for amoxicillin or co-amoxiclav, together with clarithromycin, these were named ‘trial antibiotics’. Patients were classified as having had penicillin + macrolide when these trial antibiotics were given at least 2 consecutive days in the first 4 days (regardless of other additional antibiotics). When no trial antibiotics were given, but other preventive antibiotics were given at least 2 times within the first 4 days, this was coded as ‘other antibiotics, type unknown’, since no data was present on what type of antibiotic was given when these were not the recommended trial antibiotics. Other trials: one type of antibiotic was used for all patients randomized to preventive treatment, patients were classified in the corresponding group for the antibiotic (tetracyclins, cefalosporins, fluorchinolones, penicillin + macrolide). |
| **Antibiotic therapy dose**       | When preventive antibiotic therapy was given to a patient, dose of antibiotic therapy per day in milligrams |
| **Antibiotic therapy DDD**        | When preventive antibiotic therapy was given to a patient, the dose of antibiotic therapy converted to defined daily dosis (https://www.whocc.no/atc_ddd_index/) |
| **Antibiotic therapy no of days** | The number of days that each individual patient received preventive antibiotic therapy |
| Was the total treatment with preventive antibiotic therapy administered according to study protocol / per protocol | Whether each patient separately was treated according to the study protocol of the study in which patient was included: - Kalra et al: in this trial antibiotic choice at intervention centres conformed to local antibiotic policy, but amoxicillin or co-amoxiclav, together with clarithromycin for 7 days were recommended if no restrictions applied. Treatment was considered as per protocol when the start of antibiotic therapy was initiated within 48 hours, and a patient was treated for at least 6 days of medication (day 0,2 and 4 or 2,4 and 6). If antibiotic therapy was stopped due to discharge or death before completing 6 days this was also considered treatment as per protocol. - Westendorp et al: patients who received the complete 4 days treatment or patients who did not receive the complete 4 days treatment due to death, discharge or switch of antibiotic therapy due to infection were considered as being treated per protocol. - Harms et al: patients were included in the per protocol analysis of the trial when they received the total treatment (eg patients who died within 11 days, were deblinde for medical reasons, were given less than 5 days study medication regardless of reason) were excluded. For the current analysis, per protocol was defined as total treatment but discontinuation of treatment due to death or infection was seen as treatment per protocol. - Chamorro et al: days 500 mg/100 mL levofloxacine or an identical volume of placebo (0.9% physiological serum) intravenously, treatment was withdrawn in case of diagnosis of infection or death. For the current analysis, the patients that were excluded from per protocol population in the primary article were included in de per protocol population of this meta-analysis because treatment was discontinued due to prespecified reasons, eg infection, death or fever and this was considered as per protocol in the current meta-analysis. - Chang et al: whether patients received the complete treatment. All patients did receive the complete treatment. - Fouda et al: all patients randomized to minocyclin received the complete trial treatment the per protocol population is the same as the intention to treat population. - Amiri-Nikpour et al: whether patients received the complete treatment, all patients did. - Kohler et al: patients who received the complete treatment or patients who did not receive the complete treatment due to death or discharge. - Blacker et al: patients who received the complete treatment or patients who did not receive
the complete treatment due to death or discharge.

| Time to first dose (hours) | Time in hours from stroke onset to administration of first dose of preventive antibiotic therapy.  
- Kalra et al: data unavailable. Data available is whether a patient received medication within 24h or between 24 and 48h  
- Westendorp et al: time in hours  
- Harms et al: data unavailable  
- Chamorro et al: data unavailable (was collected in trial but not able to retrieve data)  
- Chang et al: in minutes, recalculated into hours  
- Fouda et al: in minutes, recalculated into hours  
- Blacker et al: in minutes, recalculated into hours  
- Kohler: in minutes, recalculated into hours  
A2ds2Totaal Available for the following trials that included patients with ischemic stroke and in which the necessary variables were collected (age, atrial fibrillation, dysphagia, male sex, stroke severity): Kalra et al, Westendorp et al.  
ISANtot Available for the trials that collected the variables age, stroke severity and baseline modified Rankin Scale: Kalra et al, Westendorp et al, Chang et al, Blacker et al.  
PASSpneutot Available for the trials that collected age, sex, stroke severity, medical history of COPD and diabetes, baseline modified rankin scale, dysphagia: Kalra et al, Westendorp et al.  
PASSinftot Available for the trials that collected age, sex, stroke severity, medical history of diabetes, urinary catheter, baseline modified rankin scale: Westendorp et al, Chang et al. |
### Table S6. Baseline characteristics of type 1 trials

| Characteristic                          | Preventive antibiotic treatment (n=1989) | Standard care / placebo (n=1981) |
|-----------------------------------------|----------------------------------------|----------------------------------|
| Age (years)                             | 75 (65-82)                             | 76 (65-83)                       |
| Male sex (%, n/N)                       | 52 (1032/1986)                         | 52 (1031/1980)                   |
| Medical history                         |                                        |                                  |
| COPD                                    | 9 (174/1988)                           | 7 (143/1977)                     |
| Diabetes mellitus                       | 19 (383/1988)                          | 19 (379/1981)                    |
| Atrial fibrillation                     | 22 (424/1919)                          | 23 (446/1911)                    |
| Pre-stroke disability (mRS)             | 0 (0-1)                                | 0 (0-1)                          |
| Stroke severity (NIHSS)                 | 7 (4-15)                               | 7 (4-15)                         |
| Stroke type                             |                                        |                                  |
| - ischem                                | 85 (1693/1989)                         | 86 (1711/1980)                   |
| - hemorrhagic                           | 12 (229/1989)                          | 10 (191/1980)                    |
| - TIA                                   | 2 (44/1989)                            | 3 (50/1980)                      |
| - other diagnosis                       | 1 (23/1989)                            | 1 (28/1980)                      |
| Intravenous thrombolysis                | 32 (628/1950)                          | 31 (605/1938)                    |
| Dysphagia                               | 51 (922/1793)                          | 51 (918/1795)                    |
| Use of urinary cathether                | 19 (252/1304)                          | 21 (276/1305)                    |

Data in % (n/N), median with interquartile range or mean with standard deviation

COPD = chronic obstructive pulmonary disease; mRS = modified Rankin Scale; NIHSS = National Institute of Stroke Severity Scale; TIA = Transient Ischemic Attack

### Table S7. Baseline characteristics of patients included in type 2 (Minocyclin) trials

| Characteristic                          | Minocyclin (n=111) | Standard care / placebo (n=116) |
|-----------------------------------------|--------------------|---------------------------------|
| Age (years)                             | 66 (58-75)         | 68 (57-75)                      |
| Male sex (%, n/N)                       | 57 (63/111)        | 52 (60/116)                     |
| Medical history                         |                    |                                  |
| Obstructive pulmonary disease           | 3 (2/110)          | 9 (6/114)                       |
| Diabetes mellitus                       | 32 (35/110)        | 36 (41/114)                     |
| Atrial fibrillation                     | 16 (12/76)         | 21 (17/80)                      |
| Pre-stroke disability (mRS)             | 0 (0-0)            | 0 (0-0)                         |
| Stroke severity (NIHSS)                 | 9 (6-13)           | 8 (5-13)                        |
| Stroke type                             |                    |                                  |
| - ischem                                | 79 (88/111)        | 76 (88/116)                     |
| - hemorrhagic                           | 21 (23/111)        | 21 (24/116)                     |
| - TIA                                   | 0 (0/111)          | 3 (4/116)                       |
| Condition                      | % (n/N) 1 | % (n/N) 2 |
|-------------------------------|-----------|-----------|
| Intravenous thrombolysis      | 28 (31/111) | 25 (29/116) |
| Dysphagia                     | 28 (31/111) | 25 (29/116) |
| Use of urinary cathether      |           |           |

Data in % (n/N), median with interquartile range or mean with standard deviation

COPD = chronic obstructive pulmonary disease; mRS = modified Rankin Scale; NIHSS = National Institute of Stroke Severity Scale; TIA = Transient Ischemic Attack
| First Author                  | RSG (Selection) | Allocation Concealment (Selection) | Blinding of participants (Performance) | Blinding of outcome (Detection) | Attrition Bias- incomplete outcome data | Reporting Bias | Other          |
|------------------------------|-----------------|------------------------------------|----------------------------------------|---------------------------------|----------------------------------------|----------------|----------------|
| Kalra et al, 2015            | Low             | Low                                | Low                                    | Low                             | Low                                     | Low            | Low            |
| Westendorp et al, 2015       | Low             | Low                                | (Open label)- High risk for some selected outcomes | Low                             | Low                                     | Low            | Low            |
| Harms et al, 2008            | Low             | Low                                | Low                                    | Low                             | Unclear                                 | Low            | Low            |
| Chamorro et al, 2005         | Low             | Low                                | Low                                    | Low                             | Low                                     | Low            | Low            |
| Chang et al, 2017            | Low             | Low                                | Low                                    | Low                             | Low                                     | Low            | Low            |
| Fouda et al, 2017            | Unclear         | Unclear                            | High                                   | High                            | Low                                     | Low            | Low            |
| Amiri-Nikpour et al, 2015    | Unclear         | Unclear                            | High                                   | Low                             | High                                    | Low            | Low            |
| Kohler et al, 2015           | Low             | Unclear                            | (Open label)- High risk for some selected outcomes | Low                             | Low                                     | Low            | Low            |
| Blacker et al, 2013          | Unclear         | Unclear                            | Unclear                                | Unclear                         | Unclear                                 | Unclear        | Unclear        |
| Study                          | 3 Month mRS | 3-month Survival | Discharge Death | Infe-Phys | Pneu-Phy | UTI-Phy | LOS | SAE |
|-------------------------------|-------------|------------------|-----------------|-----------|----------|---------|-----|-----|
| Kalra et al, 2015             | Yes         | Yes              | ?               | Yes       | Yes      | Yes     | Yes | Yes |
| Westendorp et al, 2015        | Yes         | Yes              | Yes             | Yes       | Yes      | Yes     | Yes | Yes |
| Harms et al, 2008             | No-Only BI  | Yes-6month       | No              | Yes (<11d) | Yes      | No      | No  | Yes |
| Chamorro et al, 2005          | Yes         | Yes              | No              | Yes       | Yes      | Yes     | No  | No  |
| Chang et al, 2017             | Yes         | No               | No              | No        | No       | No      | Yes | Yes |
| Fouda et al, 2017             | Yes         | No               | No              | No        | No       | No      | No  | No  |
| Amiri-Nikpour et al, 2015     | No          | Yes              | No              | No        | No       | No      | No  | No  |
| Kohler et al, 2015            | Yes         | Yes (mRS 6)      | No              | No        | No       | No      | No  | No  |
| Blacker et al, 2013           | Yes         | Yes              | No              | No        | No       | No      | No  | No  |
Table S10. Unfavorable outcome (mRS 3-6) at 3 months in all patients per trial

| Trial          | Preventive antibiotic therapy % n/N | Standard care % n/N | OR     | 95% CI          | p-value |
|---------------|-------------------------------------|---------------------|--------|-----------------|---------|
| Kalra et al   | 81.7 (486/595)                      | 79.4 (465/586)      | 1.180  | 0.83-1.67       | 0.350   |
| Chamorro et al| 67.2 (45/67)                        | 62.3 (43/69)        | 1.041  | 0.45-2.44       | 0.926   |
| Westendorp et al | 38.5 (484/1257)         | 39.9 (502/1257)     | 0.934  | 0.77-1.13       | 0.480   |
| Harms et al   | 60 (18/30)                          | 73.3 (22/30)        | 0.516  | 0.167-1.60      | 0.250   |
| Chang et al   | 10 (1/10)                           | 10 (1/10)           | 1.040  | 0.05-21.92      | 0.980   |
| Kohler et al  | 34.1 (15/44)                        | 29.8 (14/47)        | 2.163  | 0.49-9.55       | 0.308   |
| Fouda et al*  | 66.7 (4/6)                          | 85.7 (6/7)          | 0.481  | 0.12-1.95       | 0.305   |
| Blacker et al | 17.4 (4/23)                         | 30.4 (7/23)         | 0.343  | 0.07-1.69       | 0.189   |
| **Total**     | **52.0 (1057/2032)**               | **52.2 (1060/2029)**| **0.849** | **0.60-1.19**   | **0.348** |

CI denotes confidence interval, ref denotes reference category, NA not applicable. Analysis adjusted for age and stroke severity. * for this trial only unadjusted analysis possible (number per events rule), it was excluded for the adjusted pooled analysis.

Figure S1. Forest plot for treatment effect per included trial on total range of mRS

CI denotes confidence interval, ref denotes reference category, NA not applicable. Analysis adjusted for age and stroke severity. * for this trial only unadjusted analysis possible (number per events rule), it was excluded for the adjusted pooled analysis.
| Trial                | No. of patients | OR*   | 95% CI       | p-value |
|---------------------|----------------|-------|--------------|---------|
| Kalra et al         | 1217           | 1.18  | 1.04-1.34    | 0.0129  |
| Chamorro et al      | 136            | 1.20  | 0.80-1.78    | 0.3817  |
| Westendorp et al    | 2538           | 0.99  | 0.91-1.08    | 0.8417  |
| Harms et al         | -              | -     | -            | -       |
| Pool type 1         | 3891           | 1.08  | 0.94-1.25    | 0.2703  |
| Chang et al         | 20             | 1.68  | 0.69-4.10    | 0.2568  |
| Kohler et al        | 91             | 1.63  | 1.02-2.62    | 0.0421  |
| Fouda et al**       | 13             | 0.91  | 0.23-3.58    | 0.8916  |
| Blacker et al       | 46             | 1.06  | 0.53-2.13    | 0.8639  |
| Pool type 2 adjusted| 157            | 1.46  | 1.02-2.09    | 0.0372  |
| Pool type 2 unadjusted| 170         | 1.23  | 0.88-1.74    | 0.2281  |
| All patients adjusted| 4061       | 1.13  | 0.98-1.31    | 0.0896  |
| All patients unadjusted| 4048     | 1.07  | 0.96-1.19    | 0.2226  |

CI denotes confidence interval, ref denotes reference category. * OR >1 favors control.
Adjusted for age and stroke severity (in categories)
** only unadjusted possible.
Supplementary figure 2. mRS scores at 3 months for all patients and for patients included in type 2 trials.
Table S12.

Subgroup analyses for the primary outcome of functional outcome on total range of mRS for all trials

The odds ratio represents the pooled odds ratio of the effect of antibiotic therapy vs standard therapy for each subgroup of patients within all trials. An odds ratio larger than one favors control/placebo.

| Subgroup                  | OR*  | 95% CI       | p-value | No pts | Trials included in analysis                                                                 |
|---------------------------|------|--------------|---------|--------|---------------------------------------------------------------------------------------------|
| All patients adjusted     | 1.1318 | 0.9811-1.3056 | 0.0896  | 3968   | Kalra, Westendorp, Chamorro, Chang, Blacker, Kohler.                                        |
| All patients unadjusted   | 1.0684 | 0.9606-1.1883 | 0.2226  | 4001   | All                                                                                         |
| Age >= 65                 | 1.1061 | 0.9659-1.2667 | 0.1448  | 3012   | Kalra, Westendorp, Chamorro, Blacker, Kohler.                                               |
| Age >= 65 unadjusted      | 1.051  | 0.969-1.139   | 0.2278  | 3022   | All                                                                                         |
| Age < 65                  | 1.0217 | 0.8874-1.1763 | 0.7649  | 935    | Kalra, Westendorp, Chamorro, Chang, Kohler.                                                 |
| Age < 65 unadjusted       | 1.006  | 0.875,1.157   | 0.9328  | 960    | All                                                                                         |
| Age >= 75                 | 1.1365 | 0.9628-1.3416 | 0.1305  | 2099   | Kalra, Westendorp, Chamorro, Blacker, Kohler.                                               |
| Age < 75                  | 1.0425 | 0.9418-1.1540 | 0.4223  | 1867   | Kalra, Westendorp, Chamorro, Chang, Blacker, Kohler.                                        |
| Age < 75 unadjusted       | 1.082  | 0.977-1.198   | 0.1288  | 1878   | All                                                                                         |
| Age >= 80                 | 1.2099 | 0.9396-1.5579 | 0.1397  | 1414   | Kalra, Westendorp, Chamorro, Blacker, Kohler.                                               |
| Age >= 80 unadjusted      | 1.057  | 0.939-1.189   | 0.3582  | 1414   | Kalra, Westendorp, Chamorro, Blacker, Kohler.                                               |
| Age < 80                  | 1.0206 | 0.9356-1.1133 | 0.6463  | 2560   | Kalra, Westendorp, Chamorro, Chang, Blacker, Kohler.                                        |
| Age < 80 unadjusted       | 1.035  | 0.948-1.129   | 0.4419  | 2570   | All                                                                                         |
| NIHSS 0-5                 | 0.9374 | 0.8401-1.0459 | 0.2471  | 1611   | Kalra, Westendorp, Chang, Kohler                                                           |
| NIHSS 6-42                | 1.1091 | 1.0071-1.2215 | 0.0354  | 2375   | Kalra, Westendorp, Chamorro, Blacker, Kohler.                                               |
| NIHSS 0-5 unadjusted      | 0.931  | 0.834-1.039   | 0.2001  | 1600   | Kalra, Westendorp, Chamorro, Blacker, Kohler.                                               |
| NIHSS 6-42                | 1.095  | 0.999-1.199   | 0.0513  | 2387   | All                                                                                         |
| NIHSS 0-10                | 1.0937 | 0.9152-1.3071 | 0.3245  | 2507   | Kalra, Westendorp, Chamorro, Chang, Kohler.                                                 |
| NIHSS 11-42               | 1.1513 | 1.0278-1.2896 | 0.0149  | 1480   | Kalra, Westendorp, Chamorro, Blacker, Kohler.                                               |
| NIHSS 0-10 unadjusted     | 1.013  | 0.927-1.106   | 0.7814  | 2512   | All                                                                                         |
| NIHSS 11-42 unadjusted    | 1.095  | 0.977-1.227   | 0.1183  | 1488   | All                                                                                         |
| Iv-thrombolysis yes       | 0.9510 | 0.7768-1.1643 | 0.6266  | 1262   | Kalra, Westendorp, Blacker                                                                  |
| Iv-thrombolysis no         |        | -             | -       | -      | Adjusted only data from type 1 trials                                                       |
| Iv-thrombolysis no         | 1.1070 | 0.9858-1.2432 | 0.0858  | 2155   | Kalra, Westendorp, Kohler                                                                  |
| Ischemic stroke           | 1.0931 | 0.9551-1.2509 | 0.1959  | 3385   | Kalra, Westendorp, Chamorro, Blacker, Kohler.                                               |
### Table S13.

**Subgroup analyses for the primary outcome of functional outcome on total range of mRS for type 1 trials**

The odds ratio represents the pooled odds ratio of the effect of antibiotic therapy vs standard therapy for each subgroup of patients within type 1 trials. An odds ratio larger than one favors control/placebo.

| Subgroup                  | OR     | 95% CI      | p-value  | No. of pts |
|---------------------------|--------|-------------|----------|------------|
| All patients unadjusted   | 1.0731 | 0.9783-1.4699 | 0.6602 | 420        |
| All patients adjusted     | 0.7851-1.107 | 0.5123 | 3386    | All except Chang, Fouda |
| Hemorrhagic stroke        | 1.052  | 0.853-1.299  | 0.6346 | 444        |
| Hemorrhagic stroke        | 0.9464-1.3258 | 0.1870  | 2250    | Kalra, Westendorp, Chang, Blacker, Kohler |
| Ischemic stroke unadjusted| 1.1202 | 0.9464-1.3258 | 0.8934 | 2267       |
| Time to therapy <24h      | 0.9950 | 0.9245-1.0708 | 0.8934 | 2267       |
| Time to therapy > 24h     | 0.9225 | 0.7854-1.0836 | 0.3258 | 1525       |
| Treatment per protocol    | 1.1475 | 0.8271-1.5920 | 0.4100 | 2685       |
| Placebo controlled        | 1.2643 | 0.8781-1.8204 | 0.2073 | 156        |
| Open label unadjusted     | 0.9387-1.1599 | 0.4306  | 3905    | Westendorp, Kalra, Fouda, Blacker, Kohler |
| Open label adjusted       | 0.9472-1.3233 | 0.1856  | 3892    | Westendorp, Kalra, Blacker, Kohler |

| Subgroup                  | OR     | 95% CI      | p-value  | No. of pts |
|---------------------------|--------|-------------|----------|------------|
| NIHSS 0-5                 | 0.7826-1.1425 | 0.5619  | 1554    | Kalra, Westendorp, Chamorro |
| NIHSS 6-42                | 1.0843 | 0.9396-1.2493 | 0.2703 | 3812       |
| NIHSS 0-10                | 0.9337-1.2755 | 0.2724  | 2927    | Kalra, Westendorp, Chamorro |
| NIHSS 11-24               | 1.0200 | 0.8412-1.2368 | 0.8404 | 885        |
| Age >= 65                 | 1.1091 | 0.9527-1.2911 | 0.1819 | 2049       |
| Age < 65                  | 1.0917 | 0.9178-1.2546 | 0.3767 | 3831       |
| Age >= 75                 | 1.0342 | 0.8985-1.1904 | 0.6593 | 2434       |
| Age < 80                  | 1.1096 | 0.9864-1.2482 | 0.0832 | 1383       |
| Age <= 80                 | 1.0437 | 0.9086-1.1990 | 0.5454 | 1763       |
| NIHSS 0-5                 | 0.9456 | 0.7826-1.1425 | 0.5619 | 1554       |
| NIHSS 6-42                | 1.1214 | 0.9684-1.2986 | 0.1258 | 2275       |
| NIHSS 11-24               | 1.2717 | 1.0917-1.2546 | 0.3767 | 3831       |
| Age >= 65                 | 0.9986 | 0.9127-1.0925 | 0.9754 | 2401       |
| Age < 65                  | 1.1279 | 0.9860-1.2902 | 0.0794 | 1429       |
| NIHSS 0-10                | 0.9478 | 0.7370-1.2189 | 0.6762 | 1216       |
| NIHSS 11-24               | 1.1085 | 1.0092-1.2176 | 0.0314 | 2092       |
| Ischemic stroke           | 1.0690 | 0.9270-1.2328 | 0.3590 | 3263       |

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| Hemorrhagic stroke | 1.0153 | 0.7193-1.4330 | 0.9312 | 400 | Kalra, Westendorp, Chamorro |
|-------------------|--------|----------------|--------|-----|-----------------------------|
| TIA               | 0.977  | 0.598-1.595    | 0.9257 | 86  | Westendorp                  |
| Dysphagia yes     | 1.1239 | 1.0002-1.2628  | 0.0496 | 1780| Kalra, Westendorp           |
| Dysphagia no      | 0.971  | 0.874-1.078    | 0.5791 | 1731| Westendorp                  |
| A2ds2-score > 5   | 1.0517 | 0.8676-1.2748  | 0.6077 | 1360| Kalra, Westendorp           |
| A2ds2-score <= 5  | 0.9905 | 0.8896-1.1028  | 0.8613 | 1667| Westendorp                  |
| PASS inf score>10 | 1.165  | 0.931-1.458    | 0.1810 | 376 | Westendorp                  |
| PASS inf score <=10| 0.971  | 0.880-1.072    | 0.5640 | 1949| Westendorp                  |
| ISAN-score >10    | 1.031  | 0.850-1.250    | 0.7550 | 516 | Westendorp                  |
| ISAN-score < 10   | 0.980  | 0.889-1.081    | 0.6880 | 1995| Westendorp                  |
| Time to therapy < 24h | 1.0579 | 0.8884-1.2598  | 0.5275 | 2097| Kalra, Westendorp           |
| Time to therapy >= 24h | 1.3908 | 0.7798-2.4807  | 0.2638 | 1958| Kalra, Westendorp           |
| Time to therapy:  |        |                |        |     |                             |
| - < 6             | 0.916  | 0.776-1.080    | 0.2955 | 1482| Westendorp                  |
| - 6-12            | 0.957  | 0.847-1.083    | 0.4866 | 1682| Westendorp                  |
| - 12-24           | 1.004  | 0.897-1.125    | 0.9382 | 1771| Westendorp                  |
| Type of antibiotic:|    |                |        |     |                             |
| - tetracycllin: see analysis type 2 trials | 0.991 | 0.908-1.082 | 0.8417 | 2538| Westendorp                  |
| - cephalosporin   | 1.195  | 0.802-1.782    | 0.3817 | 135 | Chamorro                    |
| - fluorchinolones | 1.132  | 0.991-1.293    | 0.0673 | 1093| Kalra                       |
| - penicillin + macrolide | 0.954 | 0.872-1.045 | 0.3110 | 2353| Westendorp                  |
| Treatment per protocol | 1.195 | 0.802-1.782 | 0.3817 | 136 | Chamorro                    |
| Placebo controlled study | 1.0728 | 0.9068-1.2691 | 0.4127 | 3755| Westendorp, Kalra           |
| Open label study  |        |                |        |     |                             |

* OR > 1 favors control/placebo

Table S14.

Subgroup analyses for the primary outcome of functional outcome on total range of mRS for type 2 trials

The odds ratio represents the pooled odds ratio of the effect of antibiotic therapy vs standard therapy for each subgroup of patients within type 2 trials. An odds ratio larger than one favors control/placebo.

| Subgroup                         | OR     | 95% CI    | p-value  | No. pts | Included trials in analysis |
|----------------------------------|--------|-----------|----------|---------|----------------------------|
| All patients adjusted            | 1.4626 | 1.0229-2.0914 | 0.0372  | 156     | Chang, Blacker and Kohler  |
| All patients unadjusted          | 1.2348 | 0.8762-1.7401 | 0.2281  | 170     | Chang, Fouda, Blacker, Kohler |
| Age >= 65                        | 1.4133 | 0.8677-2.3018 | 0.1646  | 85      | Blacker and Kohler         |
| Age >= 65 unadjusted             | 1.3551 | 0.8543-2.1494 | 0.1967  | 95      | Chang, Fouda, Blacker, Kohler |
| Age < 65                         | 1.4098 | 0.7827-2.5394 | 0.2527  | 50      | Chang and Kohler           |
| Age < 65 unadjusted              | 1.2765 | 0.7658-2.1277 | 0.3491  | 75      | Chang, Fouda, Blacker, Kohler |
| Age >= 75                        | 1.5755 | 0.6194-4.0073 | 0.3399  | 50      | Blacker and Kohler         |
| Age >= 75 unadjusted             | 1.1468 | 0.6043-2.1762 | 0.6752  | 50      | Blacker, Kohler            |
| Age < 75                         | 1.2919 | 0.8481-1.9678 | 0.2330  | 104     | Chang, Blacker and Kohler  |
| Age < 75 unadjusted              | 1.3362 | 0.8791-2.0308 | 0.1748  | 115     | Chang, Fouda, Blacker, Kohler |
| Age >= 80                        | 1.8129 | 0.2501-   | 0.5561  | 31      | Blacker, Kohler            |
| Age >= 80 unadjusted | 1.2352 | 0.5438-2.8058 | 0.6138 | 31 | Blacker, Kohler |
| Age < 80 | 1.2361 | 0.8382-1.8230 | 0.2848 | 126 | Chang, Blacker, Kohler |
| Age < 80 unadjusted | 1.2461 | 0.8454-1.8368 | 0.2664 | 136 | Chang, Fouda, Blacker, Kohler |
| NIHSS 0-5 | 1.4719 | 0.8007-2.7057 | 0.2133 | 57 | Chang, Kohler |
| NIHSS 0-5 unadjusted | 1.3872 | 0.7900-2.4359 | 0.2545 | 46 | Chang, Blacker, Kohler |
| NIHSS 6-42 | 1.1046 | 0.6938-1.7588 | 0.6749 | 100 | Chang, Blacker, Kohler |
| NIHSS 6-42 unadjusted | 1.0797 | 0.7023-1.6597 | 0.7268 | 112 | Chang, Fouda, Blacker, Kohler |
| NIHSS 0-10 | 1.3453 | 0.8207-2.2054 | 0.2395 | 106 | Chang, Blacker, Kohler |
| NIHSS 0-10 unadjusted | 1.2382 | 0.7666-2.0000 | 0.3825 | 111 | Chang, Fouda, Blacker, Kohler |
| NIHSS 11-42 | 1.8148 | 0.9370-3.5149 | 0.0772 | 51 | Chang, Blacker, Kohler |
| NIHSS 11-42 unadjusted | 1.3673 | 0.7566-2.4708 | 0.3002 | 59 | Chang, Fouda, Blacker, Kohler |
| Iv-thrombolysis yes | 1.063 | 0.531-2.125 | 0.8639 | 46 | Blacker |
| Iv-thrombolysis yes unadjusted | 0.9781 | 0.5438-1.7594 | 0.9412 | 60 | Blacker, Kohler |
| Iv-thrombolysis no | 1.460 | 0.843-2.529 | 0.1763 | 63 | Kohler |
| IV-thrombolysis no unadjusted | 1.422 | 0.814-2.487 | 0.2162 | 63 | Kohler |
| Ischemic stroke | 1.3518 | 0.8949-2.0420 | 0.1521 | 122 | Blacker, Kohler |
| Ischemic stroke unadjusted | 1.2064 | 0.8065-1.8045 | 0.3610 | 123 | Blacker, Kohler |
| Hemorrhagic stroke | 1.678 | 0.686-4.104 | 0.2568 | 20 | Chang |
| Hemorrhagic stroke unadjusted | 1.2506 | 0.6260-2.4984 | 0.5265 | 44 | Chang, Fouda, Kohler |
| Time to therapy < 24h | 1.4465 | 1.0059-2.0800 | 0.0464 | 153 | Chang, Blacker, Kohler |
| Time to therapy < 24h unadjusted | 1.2001 | 0.8497-1.6950 | 0.3005 | 170 | Chang, Fouda, Blacker, Kohler |
| Time to therapy >= 24h | NA | NA | NA | NA | NA |
| Time to therapy 0-6h | 1.049 | 0.518-2.121 | 0.8951 | 43 | Blacker |
| Time to therapy 0-6h unadjusted | 0.9348 | 0.6194-1.4108 | 0.7482 | 122 | Chang, Fouda, Blacker, Kohler |
| Time to therapy 6-12h | 2.921 | 1.661-5.136 | 0.0002 | 64 | Kohler |
| Time to therapy 6-12h unadjusted | 1.1637 | 0.4896-2.7659 | 0.7314 | 117 | Chang, Fouda, Blacker, Kohler |
| Time to therapy 12-24h | NA | NA | NA | NA | NA |
| Time to therapy 12-24h unadjusted | 1.2547 | 0.5553-2.8351 | 0.5853 | 81 | Chang, Fouda, Kohler |
| Treatment per protocol | 1.4105 | 0.9836-2.0227 | 0.0615 | 150 | Chang, Blacker, Kohler |
| Treatment per protocol unadjusted | 1.1353 | 0.8021-1.6069 | 0.4739 | 163 | Chang, Fouda, Blacker, Kohler |
| Treatment not per protocol | NA | NA | NA | NA | (only 3 patients in trial Kohler, all randomization=1) |
| Treatment not per protocol unadjusted | NA | NA | NA | NA | (only 3 patients in trial Kohler, all randomization=1) |
| Placebo controlled study | 1.678 | 0.686-4.104 | 0.2568 | 20 | Chang |
| Open label study unadjusted | 1.1975 | 0.8300-1.7278 | 0.3352 | 137 | Blacker, Kohler |
| Open label study adjusted | 1.4245 | 0.9634-2.1061 | 0.0762 | 150 | Fouda, Blacker, Kohler |

Table S15.

Subgroup analyses for the primary outcome of unfavorable functional outcome (mRS 3-6) for all trials.

The odds ratio represents the interaction effect (the effect of antibiotic treatment vs placebo/standard care compared between both subgroups) the p-value represents the p-value for interaction.
| Subgroup                  | Preventive antibiotic therapy % n/N | Standard care % n/N | OR    | 95% CI       | p-value interaction |
|--------------------------|-------------------------------------|---------------------|-------|--------------|---------------------|
| All patients unadjusted  | 52.0 (1057/2032)                    | 52.2 (1060/2029)    | 1.160 | 0.87-1.55    | 0.312               |
| All patients adjusted    | 52.0 (1057/2032)                    | 52.2 (1060/2029)    | 1.178 | 0.84-1.66    | 0.348               |
| Age per year             | NA                                  | NA                  | 1.003 | 0.99-1.02    | 0.681               |
| Age                      |                                     |                     |       |              |                     |
| - 0 - 50                 | 32.5 (37/114)                       | 30.3 (37/122)       | 1.019 | 0.89-1.17    | 0.779               |
| - 51 – 60                | 32.3 (74/229)                       | 31.4 (74/236)       |       |              |                     |
| - 61 – 70                | 38.9 (159/409)                      | 37.2 (136/366)      |       |              |                     |
| - 71 – 80                | 50.0 (313/626)                      | 53.5 (345/645)      |       |              |                     |
| - 81 – 90                | 69.7 (381/547)                      | 68.7 (388/565)      |       |              |                     |
| > 91                     | 86.9 (93/107)                       | 84.2 (80/95)        |       |              |                     |
| Age <= 65                | 32.9 (177/538)                      | 33.0 (174/528)      | 0.946 | 0.66-1.36    | 0.765               |
| Age > 65                 | 58.9 (880/1494)                     | 59.0 (886/1501)     |       |              |                     |
| Age <= 75                | 39.5 (409/1035)                     | 37.4 (378/1011)     | 1.082 | 0.9-1.49    | 0.624               |
| Age > 75                 | 65.0 (648/997)                      | 67.0 (682/1018)     |       |              |                     |
| Age <= 80                | 42.3 (583/1378)                     | 43.2 (592/1369)     | 0.842 | 0.60-1.19    | 0.328               |
| Age > 80                 | 72.5 (474/654)                      | 70.9 (468/660)      |       |              |                     |
| Stroke severity per point on NIHSS | NA                                | NA                  | 1.007 | 0.97-1.04    | 0.684               |
| Stroke severity (NIHSS): |                                     |                     |       |              |                     |
| - 0 – 5                  | 20.8 (167/801)                      | 24.8 (202/813)      | 1.201 | 0.99-1.46    | 0.063               |
| - 6 – 10                 | 49.0 (223/455)                      | 44.9 (199/443)      |       |              |                     |
| - 10 – 20                | 82.8 (466/563)                      | 81.9 (465/568)      |       |              |                     |
| - 20 - 42                | 94.3 (199/211)                      | 94.6 (192/203)      |       |              |                     |
| NIHSS 0-5                | 20.8 (167/801)                      | 24.8 (202/813)      | 0.703 | 0.51-0.97    | 0.033               |
| NIHSS 6-42               | 72.3 (888/1229)                     | 70.5 (856/1214)     |       |              |                     |
| NIHSS 0-10               | 31.1 (390/1256)                     | 31.9 (401/1256)     | 0.798 | 0.55-1.16    | 0.242               |
| NIHSS 11-42              | 85.9 (665/774)                      | 85.2 (657/771)      |       |              |                     |
| Iv-thrombolysis yes      | 47.1 (301/639)                      | 53.9 (327/607)      | 0.720 | 0.50-1.04    | 0.083               |
| Iv-thrombolysis no       | 54.2 (570/1052)                     | 51.0 (562/1101)     |       |              |                     |
| Dysphagia yes            | 78.0 (702/900)                      | 76.6 (688/898)      | 1.294 | 0.82-2.05    | 0.274               |
| Dysphagia no             | 25.1 (216/862)                      | 27.3 (237/869)      |       |              |                     |
| Stroke type              |                                     |                     |       |              |                     |
| - ischemic               | 51.7 (889/1721)                     | 52.4 (913/1741)     | 1.052 | 0.24-4.58    | 0.947               |
| - hemorrhagic            | 64.2 (158/246)                      | 62.1 (128/206)      | 1.083 | 0.23- 5.07   | 0.919               |
| - TIA                    | 11.9 (5/42)                         | 20 (10/50)          | 0.674 | 0.10-4.70    | 0.691               |
| - other diagnosis        | 21.7 (5/23)                         | 25.8 (8/31)         |       | ref          | ref                 |
| Stroke type              |                                     |                     |       |              |                     |
| - ischemic               | 51.7 (889/1721)                     | 52.4 (913/1741)     | 0.971 | 0.57-1.65    | 0.914               |
| - hemorrhagic            | 64.2 (158/246)                      | 62.1 (128/206)      |       |              |                     |
| Time to therapy <= 24h   | 48.8 (844/1731)                     | NA                  | 0.684 | 0.39-1.19    | 0.179               |
| Time to therapy > 24h    | 80.6 (104/129)                      |                     |       |              |                     |
| Time to therapy:         |                                     |                     |       |              |                     |
| Subgroup                  | Preventive antibiotic therapy % n/N | Standard care % n/N | OR     | 95% CI          | p-value interaction |
|--------------------------|-------------------------------------|---------------------|--------|-----------------|--------------------|
| All patients unadjusted  | 53 (1033/1949)                      | 53.1 (1032/1942)    | 1.160  | 0.87-1.55       | 0.312              |
| All patients adjusted    | 53 (1033/1949)                      | 53.1 (1032/1942)    | 1.177  | 0.84-1.66       | 0.348              |
| Age per year             |                                     |                     | 1.002  | 0.99-1.02       | 0.728              |
| Age                      |                                     |                     |        |                 |                    |
| - 0 - 50                 | 33.7 (35/104)                       | 31.5 (35/111)       | 1.107  | 0.89-1.17       | 0.812              |
| - 51 – 60                | 33.3 (71/213)                       | 32.3 (70/217)       |        |                 |                    |
| - 61 – 70                | 39.7 (153/385)                      | 36.8 (127/345)      |        |                 |                    |
| - 71 – 80                | 50.5 (306/606)                      | 54 (339/628)        |        |                 |                    |
| - 81 – 90                | 70.2 (375/534)                      | 69.8 (381/546)      |        |                 |                    |
| > 91                     | 86.9 (93/107)                       | 84.2 (89/95)        |        |                 |                    |
| Age <= 65                | 33.8 (168/497)                      | 33.2 (162/488)      | 1.019  | 0.70-1.48       | 0.923              |
| Age > 65                 | 59.6 (865/1452)                     | 59.8 (870/1454)     |        |                 |                    |
| Age <= 75                | 40.5 (394/974)                      | 38.0 (361/951)      | 1.097  | 0.80-1.51       | 0.570              |
| Age > 75                 | 65.5 (639/975)                      | 67.7 (671/991)      |        |                 |                    |
| Age <= 80                | 43.2 (565/1308)                     | 43.9 (571/1301)     | 0.859  | 0.61-1.22       | 0.393              |
| Age > 80                 | 73.0 (468/641)                      | 71.9 (461/641)      |        |                 |                    |
| Stroke severity per point on NIHSS | NA | NA | 1.009 | 0.98-1.04 | 0.619 |
| Stroke severity (NIHSS): |                                     |                     |        |                 |                    |
| 0 – 5                    | 21.4 (166/775)                      | 25.6 (200/781)      | 1.202  | 0.99-1.46       | 0.065              |
| 6 – 10                   | 51.1 (218/427)                      | 46.2 (193/418)      |        |                 |                    |

Subgroup analyses for the primary outcome of unfavorable functional outcome (mRS 3-6) for type 1 trials
The odds ratio represents the interaction effect (the effect of antibiotic treatment vs placebo/standard care compared between both subgroups) the p-value represents the p-value for interaction.

CI denotes confidence interval, ref denotes reference category, NA not applicable.
| Time to therapy < 24h | NA | 0.756 | 0.43-1.33 | 0.331 |
|-----------------------|----|-------|---------|-------|
| - 0-6                 | NA |       |         |       |
| - 7-12                | NA | 1.092 | 0.96-1.24 | 0.168 |
| - 13-18               | NA |       |         |       |
| - 19-24               | NA | 1.092 | 0.96-1.24 | 0.168 |
| - > 24               |     |      |         |       |
| Treatment per protocol |     |      |         |       |
| - no                 | 65.0 (191/294) | 1.247 | 0.38-4.13 | 0.718 |
| - yes                | 50.7 (835/1646) |       |         |       |
| Placebo controlled   |     |      |         |       |
| 64.9 (63/97)         | 1.178 | 0.84-1.66 | 0.347 |
| Open label           | 52.4 (970/1825) | 1.061 | 0.47-2.42 | 0.887 |

All analyses corrected for age and stroke severity.
CI denotes confidence interval, ref denotes reference category, NA not applicable.
Table S17.
Subgroup analyses for the primary outcome of unfavorable functional outcome (mRS 3-6) for type 2 trials
The odds ratio represents the interaction effect (the effect of antibiotic treatment vs placebo/standard care compared between both subgroups) the p-value represents the p-value for interaction.

| Subgroup                        | Preventive antibiotic therapy % n/N | Standard care % n/N | OR    | 95% CI   | p-value interaction |
|---------------------------------|-------------------------------------|---------------------|-------|----------|---------------------|
| All patients adjusted           | 28.9 (24/83)                        | 32.2 (28/87)        | 0.245 | 0.04-1.44 | 0.120               |
| All patients unadjusted         | 28.9 (24/83)                        | 32.2 (28/87)        | 0.481 | 0.12-1.95 | 0.305               |
| Age per year                    |                                     |                     | 1.010 | 0.93-1.10 | 0.811               |
| Age - 0 - 50                    | 20 (2/10)                           | 18.2 (2/11)         | 1.015 | 0.48-2.17 | 0.969               |
| Age - 51 - 60                   | 18.8 (3/16)                         | 21.1 (4/19)         |       |          |                     |
| Age - 61 - 70                   | 25 (6/24)                           | 42.9 (9/21)         |       |          |                     |
| Age - 71 - 80                   | 35.0 (7/20)                         | 35.3 (6/17)         |       |          |                     |
| Age - 81 - 90                   | 46.2 (6/13)                         | 36.8 (7/19)         |       |          |                     |
| Age > 91                        |                                     |                     |       |          |                     |
| Age <= 65                       | 22.0 (9/41)                         | 30.0 (12/40)        | 0.332 | 0.05-2.47 | 0.282               |
| Age > 65                        | 35.7 (15/42)                        | 34.0 (16/47)        |       |          |                     |
| Age <= 75                       | 24.6 (15/61)                        | 28.3 (17/60)        | 0.767 | 0.11-5.24 | 0.787               |
| Age > 75                        | 40.9 (9/22)                         | 40.7 (11/27)        |       |          |                     |
| Age <= 80                       | 25.7 (18/70)                        | 30.9 (21/68)        | 0.497 | 0.06-4.27 | 0.524               |
| Age > 80                        | 46.2 (6/13)                         | 36.8 (7/19)         |       |          |                     |
| Stroke severity per point on NIHSS | NA                                  | NA                  | 0.980 | 0.80-1.20 | 0.842               |
| Stroke severity (NIHSS):        |                                     |                     |       |          |                     |
| - 0 – 5                         | 3.8 (1/26)                          | 6.3 (2/32)          | 1.431 | 0.38-5.45 | 0.600               |
| - 6 – 10                        | 17.9 (5/28)                         | 24 (6/25)           |       |          |                     |
| - 10 – 20                       | 52.4 (11/21)                        | 60.0 (15/25)        |       |          |                     |
| - 20 – 42                       | 85.7 (6/7)                          | 100 (5/5)           |       |          |                     |
| NIHSS 0-5                       | 3.8 (1/26)                          | 6.3 (2/32)          | 0.891 | 0.06-13.68 | 0.934               |
| NIHSS 6-42                      | 40.4 (23/57)                        | 47.3 (26/55)        |       |          |                     |
| NIHSS 0-10                      | 11.1 (6/54)                         | 14 (8/57)           | 0.386 | 0.05-3.10 | 0.370               |
| NIHSS 11-42                     | 62.1 (18/29)                        | 66.7 (20/30)        |       |          |                     |
| Iv-thrombolysis yes             | 25.8 (8/31)                         | 37.9 (11/29)        | 0.437 | 0.01-13.41 | 0.635               |
| Iv-thrombolysis no              | 25.8 (8/31)                         | 18.8 (6/32)         |       |          |                     |
| Hemorrhagic stroke              | 25.8 (16/62)                        | 27.9 (17/61)        | 0.347 | 0.01-12.27 | 0.561               |
| ischemic stroke                 | 38.1 (8/21)                         | 47.8 (11/23)        |       |          |                     |
| Time to therapy:                |                                     |                     |       |          |                     |
| - 0-6                           | 22.5 (9/40)                         | NA                  | 2.30  | 1.11-4.76 | 0.025               |
| - 7-12                          | 19.0 (4/21)                         |                     |       |          |                     |
| - 13-18                         | 33.3 (3/9)                          |                     |       |          |                     |
| - 19-24                         | 50.0 (4/8)                          |                     |       |          |                     |
|                | 100 (1/1) |       |       |       |
|----------------|-----------|-------|-------|-------|
| Treatment per protocol |           |       |       |       |
| - no            | 50.0 (2/4) | 7.075 | 0.15-326.51 | 0.317 |
| - yes           | 26.3 (20/76) |       |       |       |
| Placebo controlled |       |       |       |       |
| Open label      | 10 (1/10) | 10 (1/10) | 1.040 | 0.05-21.92 | 0.980 |
|                 | 31.5 (23/73) | 35.1 (27/77) | 1.739 | 0.48-6.29 | 0.399 |

All analyses corrected for age and stroke severity.
CI denotes confidence interval, ref denotes reference category, NA not applicable.
## Supplementary table 18. Adverse events

| Neurological | Kalra et al, 2015 | Westendorp et al, 2015 | Harms et al, 2008 | Chamorro et al, 2005 | Chang et al, 2017 | Fouda et al, 2017* | Amiri-Nikpour et al, 2015** | Kohler et al, 2015 | Blacker et al, 2013 |
|--------------|------------------|----------------------|------------------|---------------------|-----------------|---------------------|-------------------------|------------------|---------------------|
| CT confirmed stroke extension | 23/615 (4%) vs 22/602 (4%) | - | - | - | 0 vs 0 | - | - | 1 (2.1%) vs 0 | - |
| Recurrent stroke | - | - | 1 (2.6%) vs 0 | - | 0 vs 0 | 1 vs 0 | 0 vs 0 | 0 vs 1 (2.2%) | - |
| Hemorrhagic transformation | - | - | 2 (5.1%) vs 1 (2.5%) | - | - | - | - | 1 (2.1%) vs 1 (2.1%) | - |
| Other neurologic al events | 14/615 (2%) vs 12/602 (2%) | - | - | - | - | - | - | - | - |
| General | | | | | | | | | |
| Gastrointestinal bleed | 5/615 (0.8%) vs 6/602 (1%) | - | - | - | - | - | - | - | - |
| Gastrointestinal other | - | - | 3 (7.7%) vs 4 (10%) | - | - | - | - | 1 (2.1%) vs 0 | - |
| Cardiac (MI, HF, pulmonary edema) | 15/615 (2%) vs 11/602 (2%) | - | - | 1 (2.6%) vs 1 (2.5%) | - | - | - | - | - |
| Pulmonary adverse event | - | - | 0 vs 3 (7.5%) | - | - | - | - | - | - |
| Transfer to ICU | 6/615 (1%) vs 6/602 (1%) | - | - | - | - | - | - | - | - |
| Development of antibiotic resistance | | | | | | | | | |
| Infection and/or colonization with resistant organism | 11/615 (2%) vs 14/602 (2%) (MRSA colonization) | 7 (0.6%) vs 5 (0.4%) (ceftriaxone resistant infection) | 1 vs 1 (E. coli from stool sample resistant to ciprofloxacin and moxifloxacin) vs 0 MRSA isolates (but present before start therapy) | - | 0 vs 0 | - | - | - | - |
| Side effects of medication | | | | | | | | | |
| Allergic reaction to antibiotic | - | 6 (0.5) vs 5 (0.4) | - | - | 0 vs 0 | - | - | 1 (2.1%) vs 1 (2.2%) (rash) | - |
| Diarrhoea by C difficile | 2/615 (0.3%) vs 4/602 (0.7%) | 2 (<0.2) vs 0 | - | - | 0 vs 0 | - | - | - | - |
| Phlebitis | - | 15 (1.2) vs 0 (0.7) vs 9 | - | - | - | - | - | - | - |
| Raised liver enzymes | Both: 8/615 (1%) vs 152 (12%) vs 129 (10%) | 1 (2.6%) vs 0 | - | - | - | - | 4 (8.5%) vs 2 (4.2%) | - | - |
### Oliguria or Raised Plasma Enzymes

| Abnormality | Antibiotic Therapy | Placebo/Standard Care |
|-------------|--------------------|------------------------|
| Oliguria or Raised Plasma Enzymes | 7/602 (1%) | 101 (8%) vs 112 (9%) |
| Mean creatinine at day 7 similar in both groups | - | - |

### Renal Failure

| Abnormality | Antibiotic Therapy | Placebo/Standard Care |
|-------------|--------------------|------------------------|
| Renal Failure | - | - |

### Other

| Abnormality | Antibiotic Therapy | Placebo/Standard Care |
|-------------|--------------------|------------------------|
| Miscellaneous | 6/615 (1%) vs. 8/602 (1%) | 3 (7.7%) vs 0 (nausea) | 1 (5%) vs 0 (headache) |

Data reported as no. (%) of patients randomized to antibiotic therapy vs. no. (%) patients randomized to placebo/standard care.

* Fouda et al: ‘minocycline was well tolerated’, study did not mention adverse events separately.

** Amiri-Nikpour et al: ‘During 90-day follow-up, no adverse outcomes including myocardial infarction, recurrent stroke, and mortality were observed in the both groups’, study did not mention adverse events separately.

### Supplementary table 19. Analysis of unfavorable outcome with NIHSS 5 cutoff for type 1 trials separately

| Study | NIHSS <5 | NIHSS ≥5 |
|-------|----------|----------|
| Kalra et al | | |
| mRS 0-2 | 68% (48) | 63% (42) | 12% (61) |
| mRS 3-6 | 32% (23) | 37% (25) | 88% (461) |
| | 71 | 67 | 138 |
| Chamorro et al | | |
| mRS 0-2 | 57% (4) | 57% (4) | 30% (18) |
| mRS 3-6 | 43% (3) | 43% (3) | 70% (42) |
| | 7 | 7 | 14 |
| Westendorp et al | | |
| mRS 0-2 | 80% (557) | 76% (535) | 39% (216) |
| mRS 3-6 | 20% (140) | 24% (172) | 61% (344) |
| | 697 | 707 | 140 |
| Harms et al | | |
| mRS 0-2 | - | - | 40% (12) |
| mRS 3-6 | - | - | 60% (18) |
| | - | - | 30 |

**OR p-value interaction 1.394 95% CI 0.97-2.01, p-value interaction 0.074**

**OR 1.656, 95% CI 0.75-3.67; p-value interaction 0.213**

**OR 1.642, 95% CI 0.17-16.20, p-value interaction 0.671**

**OR p-value interaction NA**
Post-hoc sample size analysis

A provisional straightforward post hoc power analysis - taking into account the ordinal nature of the modified Rankin Scale, but ignoring clustering by trial and other covariates - showed that observed total sample sizes of 2002 patients in the antibiotics groups and 1999 patients in the control groups of the meta-analysis had 80% power at a 5% two-sided significance level using a Wilcoxon (Mann-Whitney) rank sum test to detect a Cliff's delta =0.05 or higher, the difference in probability of a patient from one group having a better outcome (lower mRS score) than a patient from the other group. This detection margin of 0.05 should be considered as negligible (R-software, package ‘effsize’ 0.8.1, date 2020-10-05 by M Torchiano), suggesting sufficient power for a clinically more relevant contrast between groups.

Even for a subgroup analysis of placebo controlled trials with only sample sizes of 79 patients in the antibiotics groups and 77 patients in the control groups a similar provisional post hoc power analysis suggested 80% power at a 5% two-sided significance level using a Wilcoxon (Mann-Whitney) rank sum test to detect a Cliff's delta =0.252 or higher. This detection margin should be considered as a small effect size, suggesting sufficient power for a clinically relevant contrast between groups.